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On the morphology, anatomy, histology and function of the tarsal organ on the pedipalpi of Palystes castaneus (Sparassidae, Araneida)

by

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SYNOPSIS

Available literature is reviewed. The morphology and anatomy of the tarsal organs of *Palystes castaneus* are fully described. The course taken by the bulbus and embolus in expansion of the genital bulb is described step by step.

Histological investigations of the tarsal organs of *P. castaneus* yielded the following hitherto unreported data:

(i) The proximal and median portions of the receptaculum seminis are surrounded by two kinds of glands, namely the vesicular and syncytial glands respectively; (ii) blood vessels going to the above glandular tissue of the bulbus via the umbilical tissue (new terminology) of the retractor ligament; (iii) the absence of connections between the bulbus lacuna and the glands in the spongy tissue of the bulbus, the bulbus lacuna thus being a vascular 'cul-de-sac'; (iv) an organized arrangement of non-elastic and elastic chitinous fibres in the basal hematodocha which is responsible for the swivelling motion of the bulbus in a definite direction; (v) each spermatozoon is encapsulated.

Sperm induction is probably achieved by capillary force or resorption of secretions in the lumen of the receptaculum seminis or even more possibly a combination of both factors.

The mechanism operating ejaculation of sperm in *P. castaneus* is shown to be probably by the secretion of a colloidal substance from the vesicular glands into the fundus of the receptaculum seminis via small pores in its walls. The force pushing this secretory mass is probably the combination of hydrostatic pressure together with release of turgor pressure from the glands supplying this secretion. Stimulation of the glands is brought about by an endocrine factor probably released by a neurosecretory mechanism.

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I. INTRODUCTION

In all spiders the external opening of the reproductive organs of the male is on the ventral side of the opisthosoma near its base, in the epigastric furrow. Before pairing, males of most species of the family Sparassidae spin a small, flattish, sheet-web, called the sperm web. In this the male spider deposits a drop of seminal fluid, emitted from the sperm canal, which is then taken up and stored in a tubular cavity (receptaculum seminis) in an appendage of the last segment of the palpus, where it is retained until copulation takes place, and from which it then passes to the spermathecae of the female. The whole process is quite complex, and of course, courtship behaviour is also involved.

The aim of this investigation was mainly to study the detailed morphology and anatomy of the tarsal organ and at the same time to investigate functional aspects of its various components.

When referring to the tarsal organ of spiders, one usually means the whole tarsal segment of the pedipalpus. The term tarsal organ thus encompasses all the subdivisions into different parts of the modified tarsus. The pedipalpi are the first pair of leg-like appendages of the prosoma. Each pedipalpus consists of a coxa, trochanter, femur, patella, tibia and tarsus (Gering 1953). The segments distal to and exclusive of the coxa are referred to collectively as the palpus or palp.

The bulk of this work was conducted in 1966 as partial fulfilment for a postgraduate Honours degree in Zoology at the University of Cape Town. Investigations were carried out on specimens of *Palystes castaneus* (Latreille 1819). Follow-up work done recently at the Natal Museum to test the hypothesis put forward for the mechanism of sperm ejaculation was performed using specimens of *Palystes natalius* (Karsch 1878).

II. REVIEW OF LITERATURE

The literature pertaining to the sexual biology of spiders and its ramifications is voluminous. Specific literature relevant to each phase of this investigation is reviewed in the text.

Gering (1953) states that, 'Blackwell (1843) was one of the first to write specifically on the palpi of spiders'. Later Blackwell (1844, 1873) wrote of both the structure and function of these appendages. Westring (1861), Menge (1866), Emerton (1875, 1878, 1889), Bertkau (1875, 1876, 1878, 1884) and Van Hasselt (1876, 1877, 1886, 1888, 1889) wrote extensively on this subject. Wagner (1886) considered both the morphological features and the development of the copulatory organs of spiders, and later (1887) strongly advocated the use of the palpi as a systematic criterion.

Simon (1829–95) wrote an extensive monograph on the systematics of spiders which has a good introductory chapter on general aspects of morphology. Montgomery (1903) reported on the mating habits of spiders. Comstock (1910) brought all former information together, added the result of his own studies and produced a comprehensive treatise on the palpi of male spiders which is still a basic reference on this subject. His nomenclature has been used in the ensuing descriptions, and should form the basis of any work attempted on palpi. Szombathy (1915) produced a clear account of the structural and functional aspects of the palpus of *Agelena* and *Mygale* and was the first in interpreting the correct function of the pores in the walls of the receptaculum seminis. Gerhardt (1923, 1928, 1933) produced some excellent papers, but these could not be used much here as they mainly describe the behavioural side and positions in copulation.

Osterloh (1922) produced an excellent paper which included an analysis of the mechanics of copulation as well as an extensive morphological study of the copulatory structures. Warren (1923, 1928) wrote papers on the habits of *Palystes natalius* (1923) and an important histological investigation of the testis of this spider (1928). Barrows (1925) wrote an important paper on the origin of the tarsal organ. He showed that the receptaculum seminis was formed by a modification of the tarsal segments and the claw that migrated backwards. Bristowe (1926, 1939, 1930, 1931) published some interesting information on mating. His 1958 book is a valuable contribution but too generalized for the purpose of this investigation. Berland (1932) published a good systematic and general treatise on Arachnids as a whole. Harm (1934) worked on the structural and functional aspects of the palpus with its developmental stages. Unfortunately her section on functional aspects is not well presented and is contradictory in parts. Homann (1935, 1949) managed to measure the pressure required to raise the copulatory parts of the tarsal organ out of its resting state.

Blauvelt (1936) presented a good and well-illustrated account on the morphology of the *Linyphia* group of spiders. Crome (1951, 1954) presented some papers on the general morphology of sexual organs in spiders.

Gering's work (1953) on the structure and function of the genitalia of the American Agelenid spiders is a good piece of work, and although the agelenid palpus does not resemble that of *P. castaneus*, his clear account helped much in this investigation. Furthermore he supplies an extensive reference list. Both Merret (1963) and Van Helsdingen (1965, 1969) supply new and extensive information on the structure of male and female genital organs and their functions during courtship in Linyphiid spiders but neither of these authors supplies any information on the mechanics of sperm ejaculation. Rovner (1966, 1967), in the course of ingenious experiments with Lycosid and Linyphiid spiders, demonstrated that, contrary to earlier suggestions, sexual activities such as sperm induction and copulation in male spiders do not depend on peripheral input from the palps. Cooke's work (1966) offers a major breakthrough in our understanding of the mechanism of sperm ejaculation in *Dysdera crocata* (Dysderidae).

III. MATERIAL AND METHODS

This investigation was performed using males of *Palystes castaneus*. Live specimens were kept in glass jars, fed regularly and given water on a small cotton-wool pad.

Methods

To investigate the morphology it was found useful to sever the palpus in the coxal region of the pedipalp, then to push a syringe needle through the side of a plastic vial top (height 1 cm and diameter 3,5 cm) and then push the needle through the length of the trochanter and femur. A piece of cotton was then tied around the trochanter with the needle inside it. Alcohol could then be poured into this type of small dissecting dish to cover the palpus. This enables one to turn the needle from the outside of the vial and thus turn the palpus about its longitudinal axis for observations under the dissecting microscope, and also enables one to inject various substances into the palpus and observe through a dissecting microscope the effect of increased pressure. Figures 2, 7 and 8 were drawn using this technique. Fluids used for injecting the palps were:

(i) saline solution for fresh material;
(ii) Indian ink (black) for fresh and preserved material;
(iii) rubber latex (liquid in alkaline medium, solidifies to become rubbery in acid medium, that is 5% acetic acid) for preserved material mainly.

(i) and (ii) gave best results with fresh material. Indian ink however is not so good for cleared material. (iii) proved useful for preserved and desclerotized material.

The anatomy was investigated by means of dissections. This presented some difficulties since the average tarsal organ is 1,5 mm wide (greatest width), 4,5 mm long and the whole palpus, including the tarsal organ, is 11 mm. Furthermore, portions of the tarsal organ are heavily sclerotized, making cutting and holding in place very difficult. Fine scalpels were made out of dissecting needles that had been flattened down at their tips and sharpened on a small oilstone under a dissecting microscope. Some palpi were also desclerotized by boiling in 10% KOH for a few minutes. The palpi thus treated were used for injecting and dissecting techniques. The KOH method has the drawback of destroying all internal tissues, leaving only cuticular membranes and sclerites. This is thus only useful in dissections when tracing the pattern of the receptaculum seminis. Palpi for dissection (especially non-desclerotized ones) were inserted in a small amount of molten wax at the bottom of a miniature dissecting dish. When the wax had cooled and the palpus was firmly held, the wax covering the tarsal organ was removed under the dissecting microscope and the dissection performed under alcohol.

Serial sections were also prepared for anatomical and histological purposes. Sectioning and the preparation of material followed standard procedure and need not be described here, except where special methods were used. When cutting sections, whole palpi were used. The cuticle of the tarsal organ and the tibia of the palpus ranges from flexible non-sclerotized to heavily sclerotized cuticle. The latter caused much trouble in sectioning.

All material used was fixed in alcoholic Bouin, washed in alcohol several times and then put in several changes of lithium carbonate to remove remaining acetic and picric acids. The material was then softened in diaphanol as advised by Pantin (1964). For subsequent treatment two methods were used.

Method I: Peterfi's method of preimpregnation with celloidin as described by Pantin (1964). Briefly, after dehydration it consists in passing the material through methyl benzoate celloidin with several changes, then benzene, then benzene and paraffin and finally embedding in paraffin in the usual way. This method proved to be unsatisfactory for the heavily sclerotized portions and the material was ruined beyond use when sectioning.

Method II: Wigglesworth's (1959) double embedding method in agar and ester wax. Briefly, after diaphanol treatment, it consists in first embedding the material in an agar block then cellosolve and ester wax and finally several changes of ester wax and then embedding in ester wax. This method gave a fair amount of success, inasmuch as some of the material sectioned could be used, but many sections were also badly damaged.

Three different types of stains were used. These were:

(i) *Mann's methyl blue-eosin* staining procedure as described by Pantin (1966). This gave very good results and can be recommended.

(ii) *Mallory's triple stain* method as described by Pantin (1966), Carleton (1938) and Humason (1962). The procedure is more lengthy and tricky. Times have to be worked out carefully, but once this has been done, it gives excellent results and better differentiation of the various tissues than Mann's procedure. This stain was used in an attempt to establish the presence of collagen. No collagen was proved to be present.

(iii) *Orcein and Van Gieson stain* to show the presence of collagen and elastin simultaneously. Much trouble was experienced in this procedure, even though the stains were freshly prepared. Final results however showed faintly positive results for elastic fibres. This is the method described by Carleton (1938).

These stains were used to stain three sets of longitudinal and three sets of transverse sections. Altogether 120 slides were thus prepared and stained.

IV. TERMINOLOGY

The meanings of certain terms used in this paper need to be clarified.

When considering the various components of the tarsal organ, one of them, namely the bulbus, is pushed out from the tarsal organ and swivelled round at an angle of more than 180°. This will change its orientation. It is important to bear in mind that the terms defined below apply to resting conditions, when describing, say, a region of the bulbus.

Dorsal: refers to the dorsal side when the longitudinal axis of the palpus is parallel to that of the animal's body. In such event the plane of the dorsal side of the palpi is parallel to that of the dorsal side of the body. When the palpi are lowered or moved about, the portion described as the dorsal side will still be referred to as such.

Ventral: the same applies to this term except that conditions are reversed.

Ectal: refers to the side of a component that in normal resting condition faces the outer lateral region of the body, e.g. the tibial apophysis labelled in fig. 1 is on the ectal side of the right palpus.

Ental: is the opposite of ectal and refers to the side of a component normally facing inwardly at rest.

When the bulbus swivels round at an angle of 180° the side that is the ectal side at rest now faces 'entally' with respect to the body, but it is still the ectal side of the bulbus and will be referred to as such.

In order further to avoid confusion, all tarsal organs described in the text will be those of the right palpi, except where the contrary is stated.

V. RESULTS

1. *Morphology of the tarsal organ of* P. castaneus

The terminology used is that employed by most authors since and including Comstock's work (1910). Where terminology varies due to language differences this is pointed out.

Figure 1 represents a portion of the right palpus seen ventrally. The segment beyond the distal intersegmental membrane of the tibia is the tarsal organ.

In *P. castaneus* the tarsal organ is broadly divisible into the following main, distinct components.

(1) The bulbus (figs 1 and 2).
(2) The basal hematodocha (figs 2 and 5) or membranous connection between (1) and (3).
(3) The cymbium (figs 1 and 5).

Note: (1) and (2) considered together are often called the genital bulb.

(1) *The bulbus*

It is broadly speaking, an oval-shaped structure. It forms a distinct part of the tarsal organ and is the portion that bears the intromittent organ or embolus. It also encloses the receptaculum seminis. The bulbus is almost completely sclerotized except for a few structures. Most authors agree that the bulbus is formed of distinct sclerites. The number of these sclerites varies considerably amongst different spiders. Sclerites may also give rise to various accessory organs such as apophyses of different shapes and functions.

In *P. castaneus* the bulbus is divided into two main components which are not clearly distinguishable at first. These two components are the tegulum and subtegulum (see fig. 2.).

(i) The tegulum

It is the only portion of the bulbus that can be seen in fig. 1 and it forms two-thirds of the bulbus. This is well seen in fig. 2. The tegulum, at rest, fits closely against the subtegulum and the two are joined all around by a tough, short, non-sclerotized, membrane. The membrane is shorter on the ental side than on the ectal side and is referred to as the *hematodochula* (Osterloh 1922) or median hematodocha (Comstock 1910, and Gering 1953). The hematodochula is also well seen in figs 9 and 10.

The tegulum itself is composed of various projecting parts. Most authors refer to these as the apical projections or apophyses. In my opinion the term apical is somewhat confusing, as we are dealing here with the ventral side of a structure. It is better to refer to them as distal projections. These are:

(*a*) The embolus: In certain spiders, e.g. Agelenidae, this is composed of a proximal segment, the radix and a distal one, the stipes. This is not the case in Sparassidae. The region where a radix is present in other spiders is labelled 'radix region' in fig. 1. The ventral proximal end of the embolus is moderately sclerotized and is joined synarthrodially with the tectal

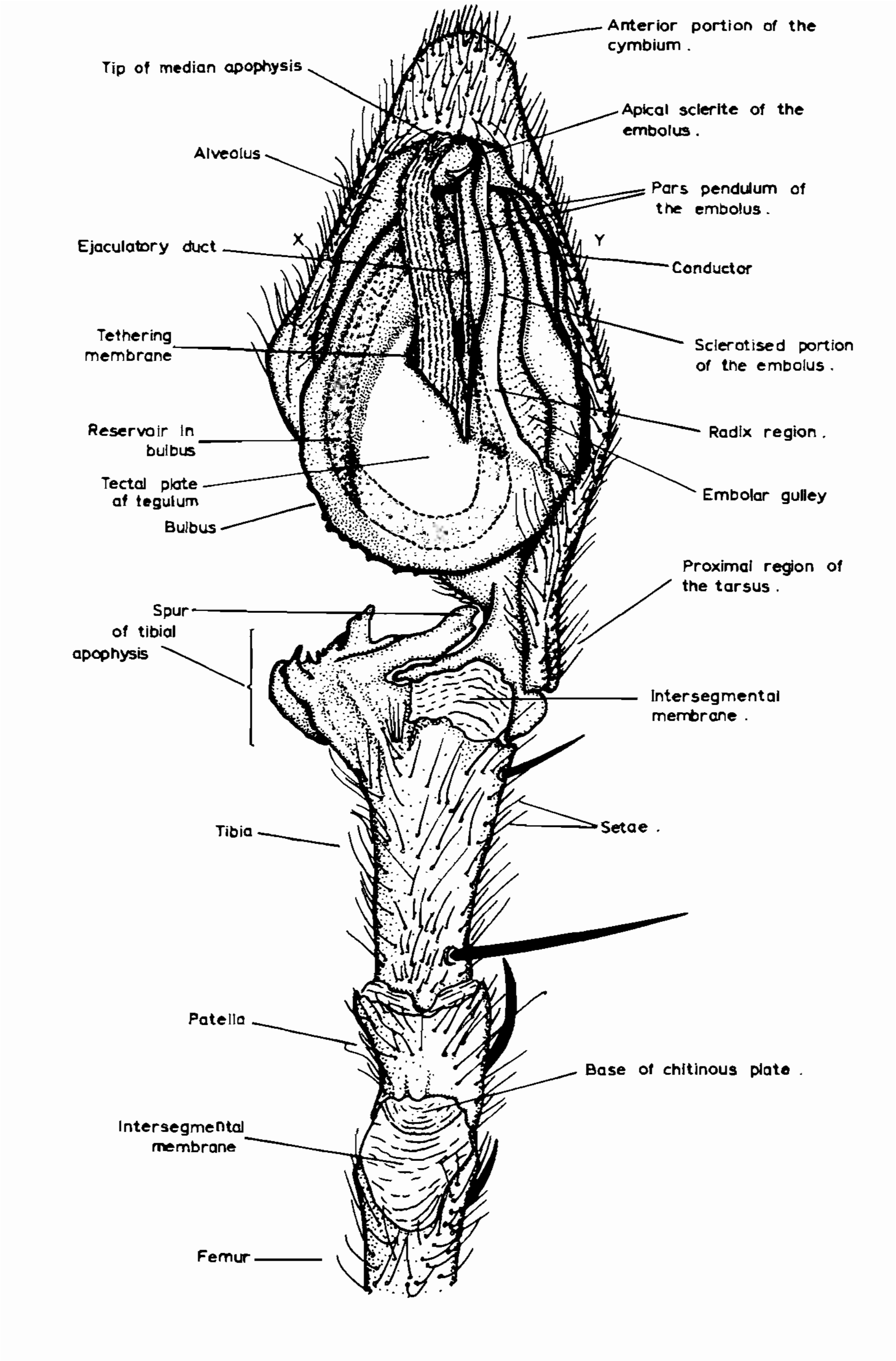

Fig. 1. Ventral view of right male pedipalp of *P. castaneus*. Ectal side on left-hand side of page.

plate of the tegulum. Most of the ventral portion of the embolus is sclerotized and the distal part has a sickle-shaped sclerite (figs 1, 2 and 3) that is fused to the ventral, sclerotized portion of the embolus. The ejaculatory part of the receptaculum seminis ends in the median ental tip of this sclerite (figs 1, 2 and 3). The pars pendulum, or pendular membrane (figs 1, 2 and 3), is the semi-membranous part of the embolus which runs along its length deep to the sclerotized portion as seen in figs 1 and 2. The pars pendulum is folded at rest and is attached to the region of the embolar gulley of the conductor (figs 1 and 2). In expanded genital bulbs, the pars pendulum becomes more readily visible when the sclerotized portion of the embolus is forced away slightly as in fig. 8. The ejaculatory duct of the receptaculum seminis can be traced through the pars pendulum together with the distal end of the reservoir of the receptaculum seminis. The primary function of the embolus, especially the embolus tip, is to bring the ejaculatory duct into the genital tract of the female for deposition of the seminal fluid.

(*b*) The conductor of the embolus: Its main function appears to be that of protecting the pendular membrane. This is done mainly by its embolar gulley where the embolus comes to rest when inactive. The term *embolar gulley* was derived from its function by myself as no adequate term could be found in the literature.

(*c*) The median apophysis: This resembles a long stocking in shape and emerges from the tectal plate region of the tegulum (fig. 1). The tip of the median apophysis is spatula-shaped and rests over the apical sclerite of the embolus (fig. 2). Its function is probably to protect the embolus tip or the opening of the ejaculatory duct. The median apophysis is membranous and has many crinkles in it. The proximal end of this apophysis is connected to the tegulum by means of a tough membrane called the tethering membrane. The tethering membrane is also connected to a portion of the proximal part of the pendular membrane.

(ii) The subtegulum

Whereas the tegulum is like a complete dome covering the subtegulum, and attached to it by the hematodochula, the subtegulum is in the shape of a horseshoe with a more or less convex plate (looking at the dorsal side of the bulbus) stretching across it. The plate is termed the *basal plate* (fig. 4) and has a gulley type of invagination called the condyle. As the subtegulum is not a complete ring, it will become apparent later that in fact the hematoduchula is merely a continuation of the basal hematodocha that extends between the tegulum and subtegulum. The subtegulum has its thicker and bulkiest portion on the ental side, which I have termed the *ental boss of the subtegulum*, as no suitable term could be found in the literature (see fig. 2). The subtegular boss is surmounted by a small ridge which I have called the *ental ridge of the subtegulum* (fig. 2). As one proceeds anteriorly along the subtegulum (away from the ental boss), the subtegular boss and the subtegular ridge can be seen to taper. On reaching the ectal side, the ridge tapers completely and ends to form the other side of the 'horseshoe' where the basal plate meets it (fig. 4).

(2) *The basal hematodocha*

Gering (1953) states that 'the basal hematodocha, petiole and subtegulum are referred to collectively as the basal division of the genital bulb'. This is correct for Agelenids but not for *P. castaneus*. The subtegulum is more a part of the bulbus than of the hemato-

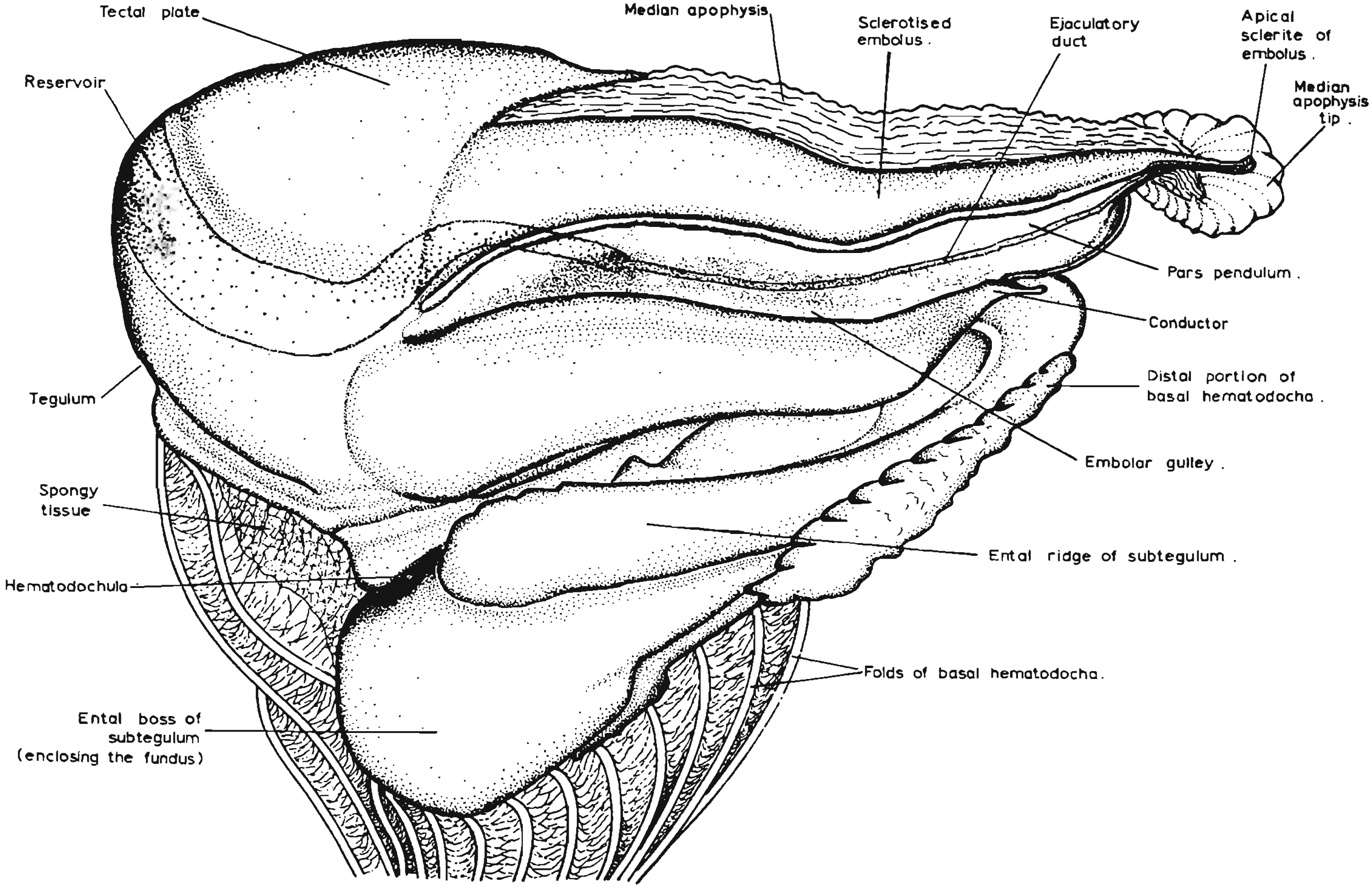

Fig. 2. Lateral view of ental side of bulbus and distal portion of basal hematodocha of male tarsal organ of *P. castaneus.*

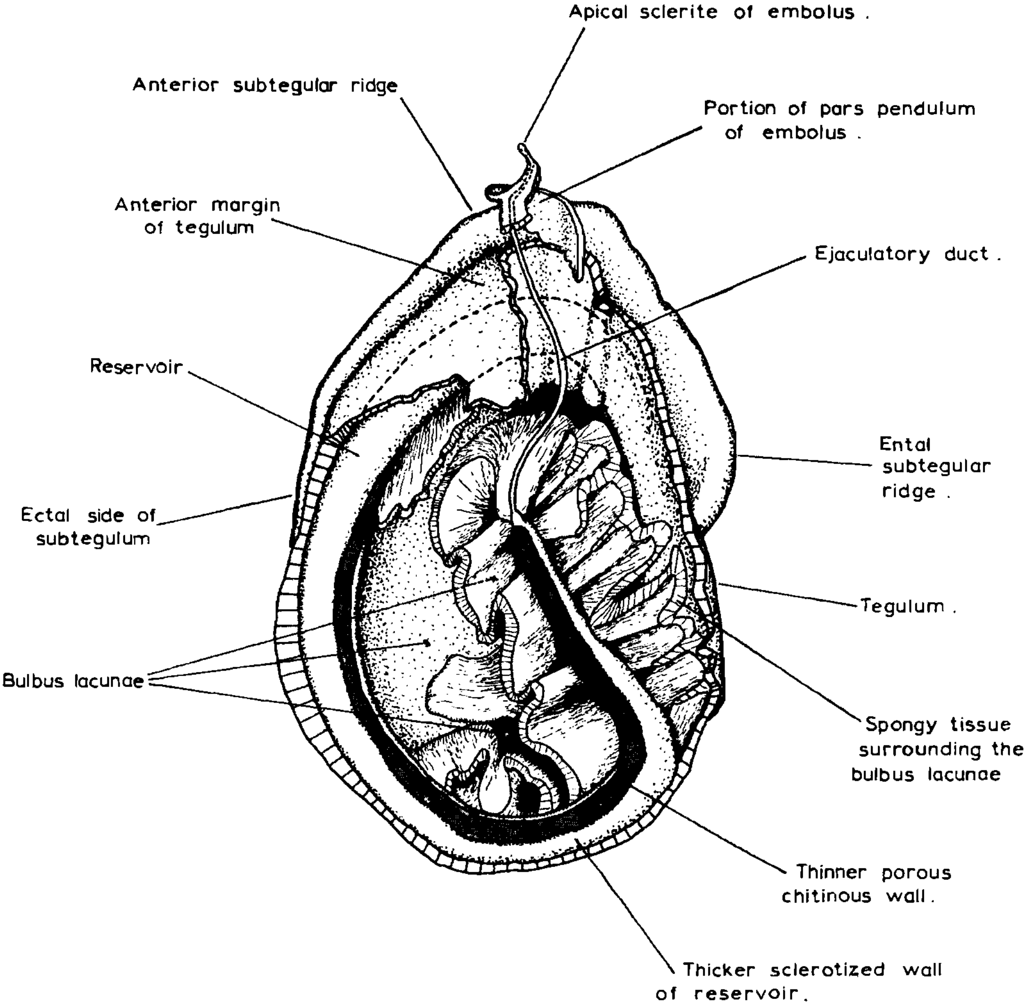
Apical sclerite of embolus .
Anterior subtegular ridge
Portion of pars pendulum of embolus .
Anterior margin of tegulum
Ejaculatory duct .
Reservoir
Ental subtegular ridge .
Ectal side of subtegulum
Tegulum .
Bulbus lacunae
Spongy tissue surrounding the bulbus lacunae
Thinner porous chitinous wall .
Thicker sclerotized wall of reservoir .

Fig. 3. Dissected right bulbus of male tarsal organ of *P. castaneus*. Orientation same as fig. 1. Tectal plate, some of spongy tissue and glandular tissue removed to show ejaculatory duct and reservoir of receptaculum seminis.

docha. The basal hematodocha, figs 2 and 5, is a membranous sac-like structure, with its proximal end connected to the cymbium inside the alveolus (fig. 5) and its distal end connected to the subtegulum and posterior portion of the tegulum (fig. 2). This latter connection is due to the fact that the subtegulum does not form a complete ring.

Wagner (1887) first used the term 'haematodocha' because this structure was distended with 'blood' during copulation. Comstock (1910) showed that Menge's term 'spiral muscle' was inappropriate for the basal hematodocha because of the total absence of muscles from the genital bulb. The basal hematodocha is inconspicuous or completely concealed by other

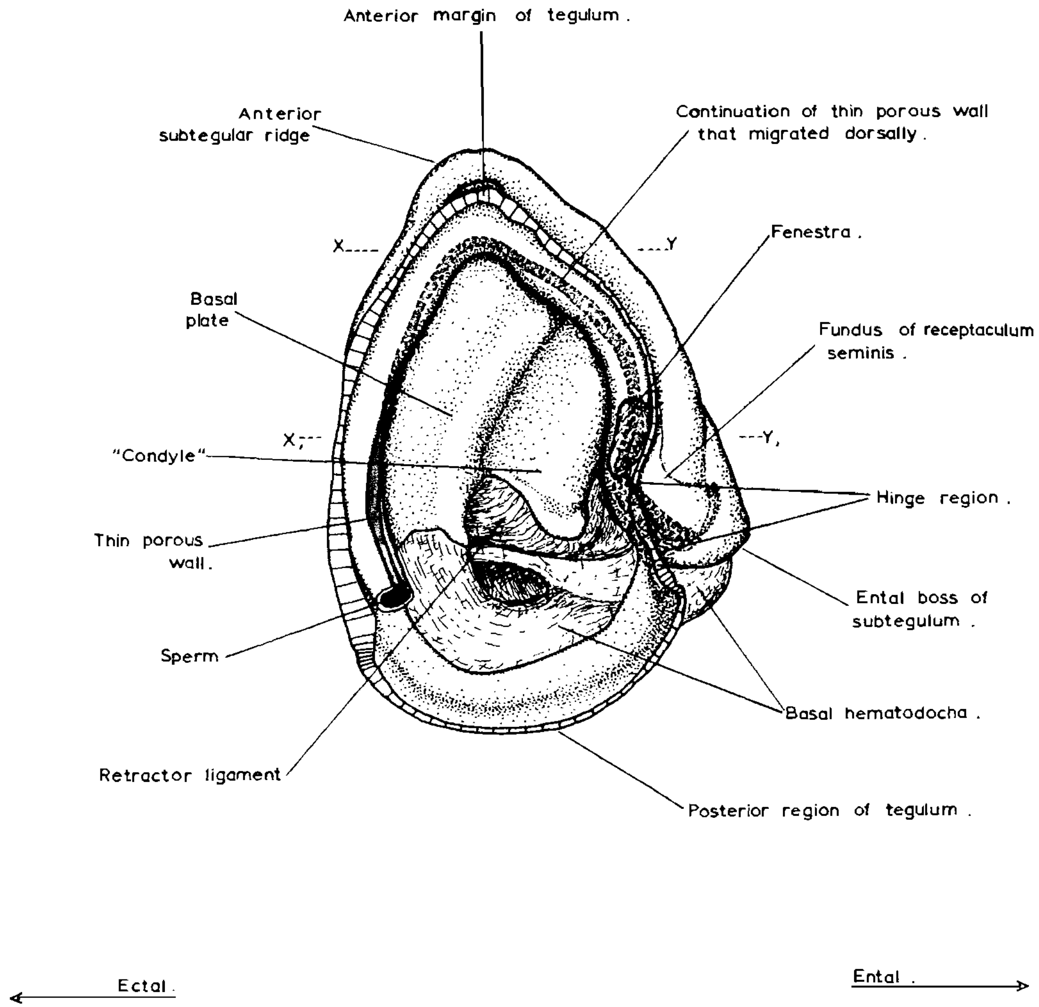
Anterior margin of tegulum.
Anterior subtegular ridge
Continuation of thin porous wall that migrated dorsally.
X
Y
Fenestra.
Basal plate
Fundus of receptaculum seminis.
"Condyle"
X,
Y,
Hinge region.
Thin porous wall.
Ental boss of subtegulum.
Sperm
Basal hematodocha.
Retractor ligament
Posterior region of tegulum.
Ectal.
Ental.

Fig. 4. Same as in fig. 3 but with most of tegulum removed to show basal plate of subtegulum and path of fundus of receptaculum seminis. Ejaculatory duct, distal portion of reservoir, all of spongy tissue and glands removed.

parts of the bulb, when the tarsal organ is at rest, but it becomes very conspicuous in the expanded bulb (figs 7 and 8). When not expanded, this membranous tube is folded and twisted upon itself. Figures 2 and 5 show the fold thus formed.

(3) *The cymbium*

Comstock (1910) adopted Menge's (1866) term 'cymbium', because of general usage. Osterloh (1922) calls it the 'Schiffchen' or ship. This latter term, although out of use, accurately describes the general shape of the cymbium (see figs 1, 5 and 8). The *alveolus* is the concavity in the ventral portion of the cymbium (figs 1, 5 and 7). Comstock adapted this term from Menge (1886). Usually the genital bulb or bulbus with basal hematodocha lie almost wholly within the alveolus. The cymbium, except for a thicker 'knob' (fig. 16) on the ectal side, consists of a thin, sclerotized layer of cuticle and is abundantly covered with short, variously developed setae (fig. 1).

The cymbium, with its alveolus, appears to serve a dual purpose:

(*a*) it is the foundation for the genital bulb and its activity during copulation, and,
(*b*) it protects the hematodocha of the unexpanded genital bulb.

The term *petiole* was proposed by Chamberlin (1904). The petiole is a strap-like, flattish, triangular and concave sclerotized segment found within the alveolus (figs 5, 8, 9 and 16), and closely associated with the proximal end of the basal hematodocha. It is located on the ectal side of the alveolus in the inactive tarsal organ. The triangular petiole has a fairly thick and strong, chitinized ligament that attaches its broad base to the inner ectal wall of the cymbium and runs beneath it and on its sides, towards the inner margins of the subtegular boss and part of the basal plate (figs 5 and 16). Only a few authors have mentioned this ligament which they call the retractor ligament. In fact their terminology

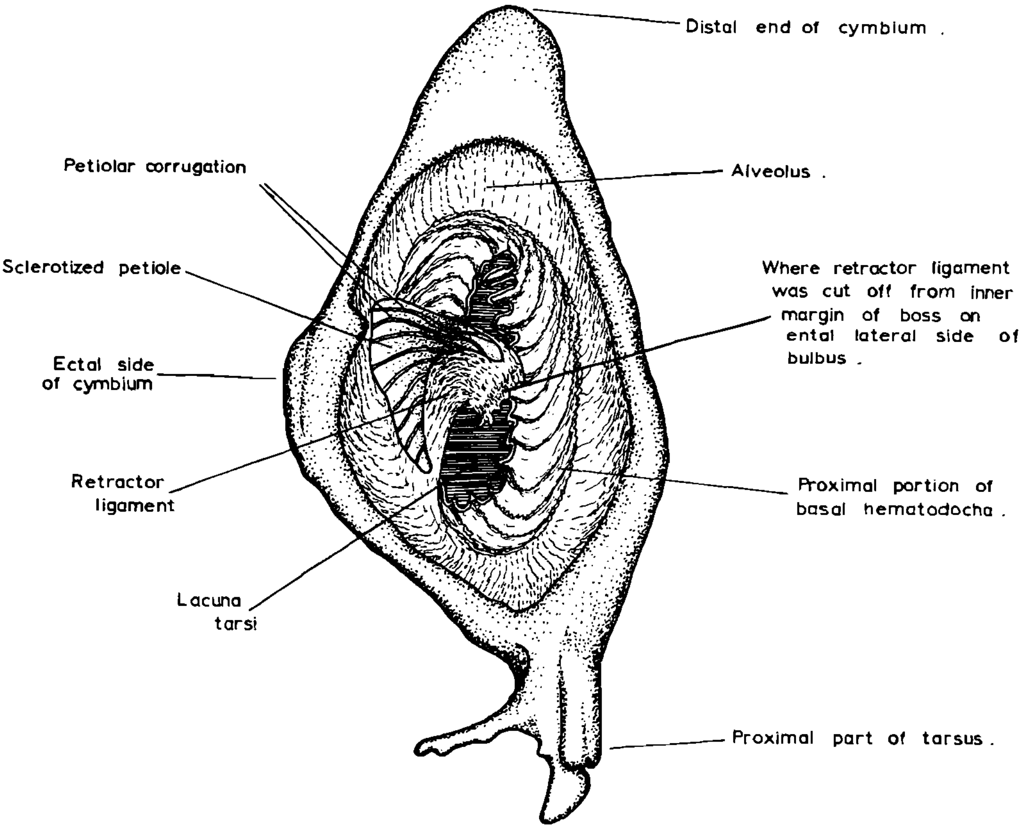
Distal end of cymbium .
Petiolar corrugation
Alveolus .
Sclerotized petiole
Where retractor ligament was cut off from inner margin of boss on ental lateral side of bulbus .
Ectal side of cymbium
Retractor ligament
Proximal portion of basal hematodocha .
Lacuna tarsi
Proximal part of tarsus .

Fig. 5. Ventral view of right cymbium of *P. castaneus*. Orientation same as in fig. 1. Bulbus and distal portion of basal hematodocha removed to show alveolus, petiole and retractor ligament.

refers to the petiole-ligament entity (Comstock 1910, and Osterloh 1922). I recognize a distinction between the ligamentous and petiolar portion of the retractor ligament. The sclerotized petiole is stiff and can be bent only to a small extent. The ligamentous part of the retractor ligament can be bent and slightly twisted, but as it is chitinous it cannot be flattened easily. This will become relevant in later discussions. The petiole, when viewed as in fig. 5, has what I have termed *petiolar corrugations* on its dorsal (inner) side. These are formed by small indentations or gullies on the inner side of the petiole. A possible function of these will be discussed later.

The palpal segments proximal to the tarsal organ do not really form part of this investigation but their morphology will be considered *en passant* as two things are worth mentioning. These are (i) *the tibial apophysis*, which is large and very noticeable even with the naked eye (fig. 1). The tibial spur is very important in the copulation of Sparassidae as pointed out by Bristowe (1926, 1958): 'The male inserts his tibial spur into one of the two chitinous pockets in the female's body just behind her epigyne and this locks the palp to resist the pressure set up by the expansion and tension of the palpal organ' (Bristowe 1958). The tibial spur is also a good means of differentiating *P. castaneus* from *P. natalius*, at a first glance, as the latter has only a long conical spur and no ectal boss on the apophysis as in *P. castaneus* (fig. 7).

(ii) *The chitinous plate* whose base can be seen by transparency through the intersegmental membrane between the patella and the femur (fig. 1). A special muscle (observed in serial section), the levator of the chitinous plate, as described by Parry (1957, 1959, 1960), can draw the plate which normally lies in a horizontal position, into a vertical position and thus largely limit the increased haemolymphatic pressures. The importance of this device is obvious in that it will give the spider better control over the palpi and prevent expansion of the genital bulb every time the spider raises its haemolymphatic pressure to work its legs in walking and jumping. Parry also described a similar chitinous plate in the legs of jumping spiders.

Having considered the morphology of the different components of the tarsal organ, let us see how these parts are affected during expansion of the genital bulb.

2. *The expansion of the genital bulb*

As this process could not be observed *in situ* during copulation, the effect of increased haemolymphatic pressure on the movements of the genital bulb had to be investigated by artificially-created increased pressures. This was done by injecting various fluids in the palpar lumen, as described earlier.

Haemolymphatic activation of the genital bulb is the only hypothesis that is supported by both anatomical and experimental investigations of other workers. Homann (1935) found that the genital bulb could be inflated with water under 1,5 atmospheres of pressure. He stated that the action thus induced closely approximated normal inflation of the hematodocha during copulation. I found that artificially induced pressures (by injections) elicited the same response. Gering (1953), describing copulation in Agelenidae, divides the sequence of events in the inflation of the hematodocha into two parts: (i) Initial inflation of the hematodocha, which forces the genital bulb out of the alveolus, rotates it and places it in a favourable position for the insertion of the embolus and associated events. (ii) Maximum

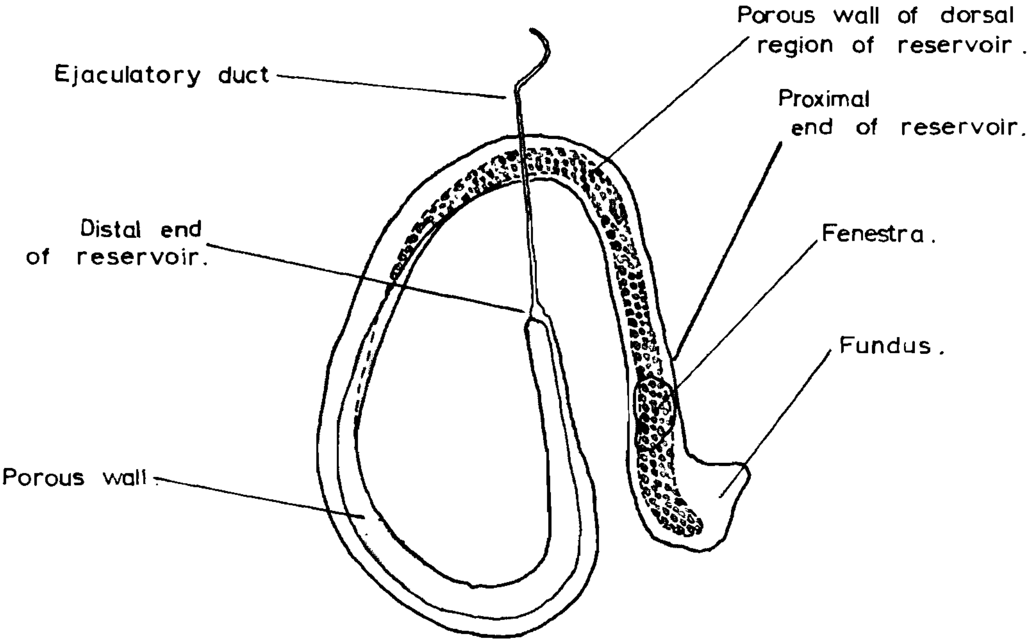
Porous wall of dorsal region of reservoir.
Ejaculatory duct
Proximal end of reservoir.
Distal end of reservoir.
Fenestra.
Fundus.
Porous wall

Fig. 6. Semi-diagrammatic representation of receptaculum seminis showing various regions.

distension of the hematodocha, which is achieved only after the locking of the palpus is completed. He observed that: 'during this period, the hematodocha pulsates quite constantly during any single insertion. Maximum distension is actually a dynamic state in which complete inflation of the hematodocha is maintained only momentarily, followed by a slow, slight deflation. The pulsating erections of the spines of the male are synchronous with the hematodochal palpitations. Ejaculation, in all probability, occurs during the maximum distension of the hematodochae.'

Figures 7 and 8 show the relative positions assumed by the bulbus and hematodocha after maximal artificial inflation. Using fig. 7, the sequence of events is as follows:

(i) Initial inflation of the basal hematodocha forces the genital bulb out of the alveolus, slightly ectad and towards the reader in fig. 7, i.e. from 0 to 1 along the heavy broken line. Note: the broken line traces the arc followed by the embolus tip during inflation.

(ii) Further increase in pressure causes ectal swivelling and additional upward movement of the embolus towards the viewer of fig. 7, that is the embolus tip describes an arc from 1 to 2 along the heavy broken line.

(iii) Additional increase in pressure causes the embolus tip to dip towards and beyond the ectal boss of the tibial apophysis. At the same time the ectal margin of the tegulum is raised towards the viewer of fig. 7, that is the embolus tip moved along arc 2 to 3 of the heavy broken line.

(iv) Final and maximal increase in pressure (beyond which the hematodocha was found to burst) caused the embolus tip to move well beyond the tibial apophysis to the level of the dorsal intersegmental membrane between the cymbium and tibia (this is better seen in fig. 8). This will be the path followed by the embolus tip beyond point 3 of the heavy broken line in fig. 7.

It can be seen from fig. 8 that the ental side of the subtegulum now rests against the postero-ectal side of the cymbium. Part of the petiole can be seen protruding over the ectal

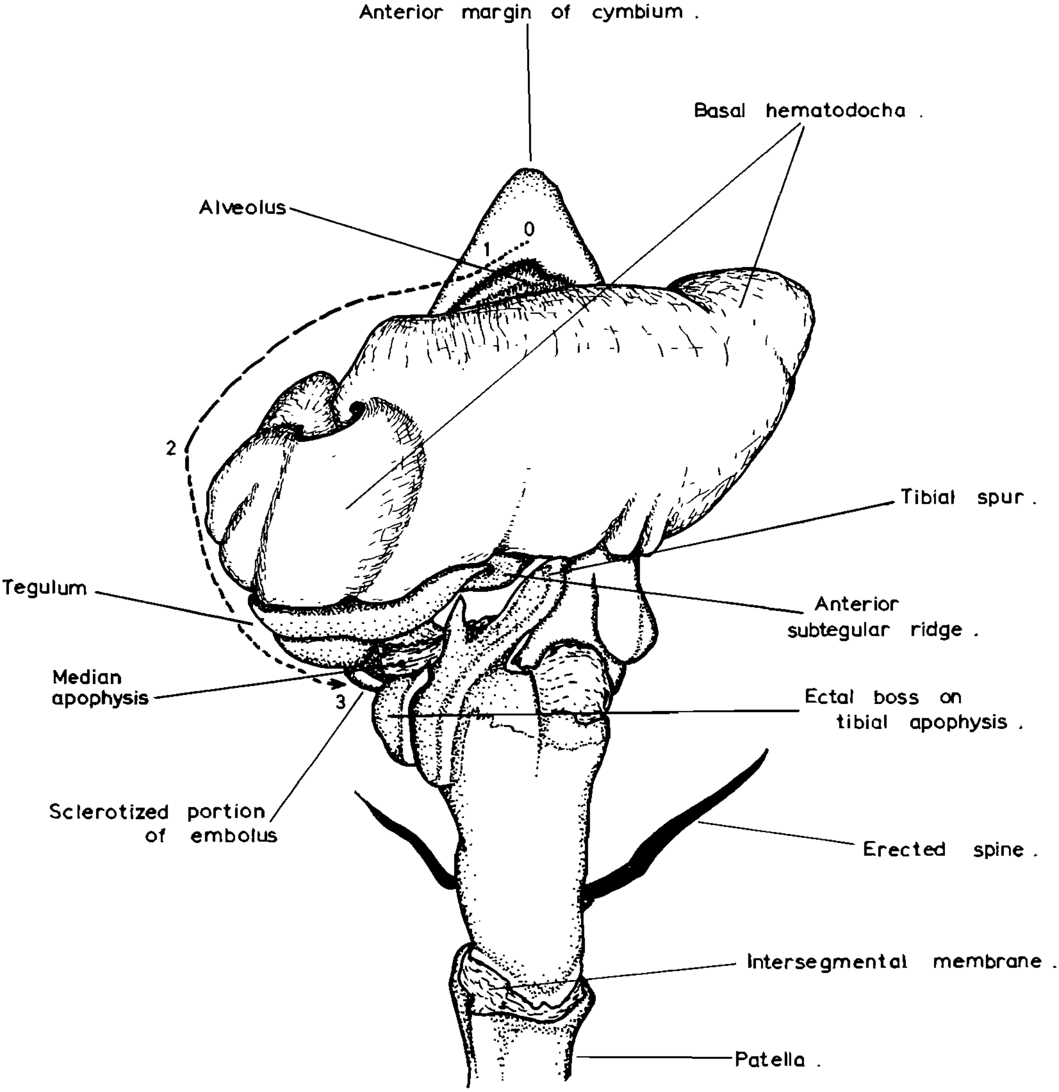
Anterior margin of cymbium .
Basal hematodocha .
Alveolus
0
1
2
Tibial spur .
Tegulum
Anterior subtegular ridge .
Median apophysis
3
Ectal boss on tibial apophysis .
Sclerotized portion of embolus
Erected spine .
Intersegmental membrane .
Patella .

Fig. 7. Right tarsal organ of *P. castaneus*. Orientation of cymbium is approximately the same as in fig. 1. This ventral view shows disposition of the various parts of the bulbus at maximal expansion of the hematodocha as when caused by raised haemolymphatic pressure. Vestiture, except spines, omitted. Expansion in this instance was caused by injecting rubber latex (see text).

rim of the cymbium. One can also see in fig. 8 that the sclerotized portion of the embolus has been pushed away from the embolar gully as mentioned earlier.

In the description of the tegulum we saw that this structure is connected to the subtegulum by the short hematodochula. It was also noted that the ental hematodochula is shorter than the ectal position. It should be mentioned, although this is probably of no importance in the case of *P. castaneus*, that at maximal expansion this causes the ectal margin of the tegulum to be slightly separated from the subtegular ridge.

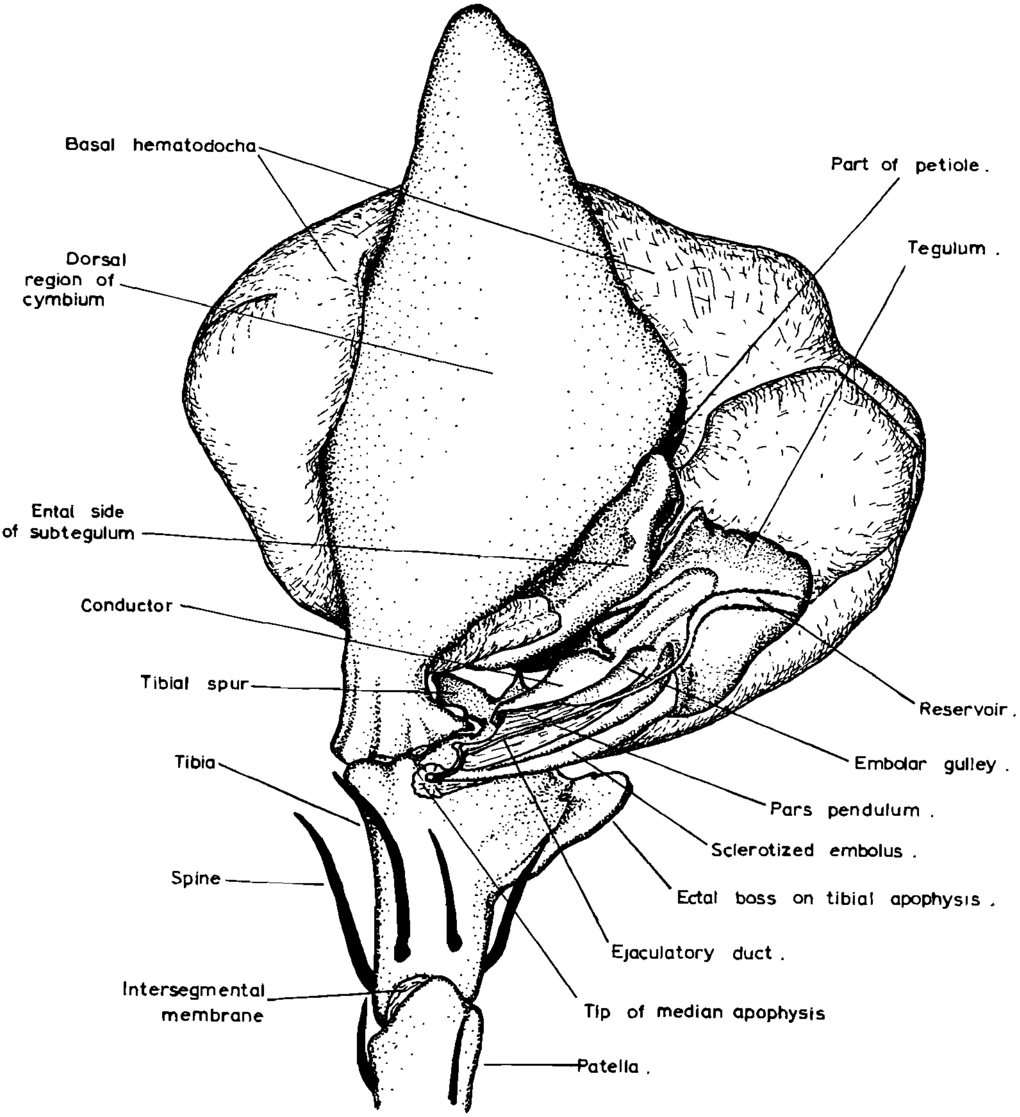

Fig. 8. Right tarsal organ of *P. castaneus*, seen in dorsal view, to show various parts at maximal expansion of the hematodocha. Vestiture, except spines, omitted. Expansion caused by rubber latex injection.

In fig. 4, a region between the ental side of the tegulum and the subtegulum has been labelled the hinge region. This is because it was observed that, at maximal expansion, the whole tegulum hinges on this portion. This is illustrated diagrammatically in fig. 16 where one can see that the distance X–Y is slightly greater in fig. 16*b* than in fig. 16*a*. However this movement is only slight. It is thought that this movement is facilitated by the presence of the fenestra, which occurs more or less in the region where the reservoir ends in the tegulum (that is proximal end of reservoir) and the fundus starts in the subtegulum (that is distal end of the fundus).

The fenestra thus partly acts as a hinge and hence prevents too much tension being exerted on the receptaculum seminis. How this motion may affect the sperm mass stored in the receptaculum seminis will be referred to later.

3. *Anatomy of the tarsal organ*

The anatomy was worked out from dissections under the dissecting microscope and serial sectioning.

(1) *The bulbus*

Gering (1953: 13–14) stated: 'The receptaculum seminis (vesicula seminis), consisting of the fundus, reservoir and the ejaculatory duct, is the only internal structure found in the genital bulb.'

However, upon removing the tectal plate of the bulbus, I found a mass of what has been termed the spongy tissue (fig. 3). This was found to completely surround the receptaculum seminis except in the distal portion. As will be shown later, this spongy tissue was found to be composed of glandular and connective tissues, small blood vessels and compact connective tissue in certain regions. Szombathy (1915), Osterloh (1922) and Harm (1934) also reported the presence of this tissue. One may point out that Gering investigated Agelenidae and that perhaps these tissues are not present in that family. This is not the case, however, as Szombathy and Osterloh found such tissues in the bulbus of *Agelena similis*. Yet Gering had read Osterloh's paper as he says on p. 14: 'Osterloh (1922) presented evidence of the role of a hydraulic mechanism in the movement of the copulatory apparatus.' Why Gering chose to ignore Szombathy's, Osterloh's and Harm's findings is rather puzzling. It may be that he did not accept the findings of these three authors.

In the course of this investigation, three main types of structures were found in the bulbus, namely (i) the receptaculum seminis, (ii) the spongy tissue mass, (iii) a continuation of the hematodocha into the bulbus, termed the inner bulbar hematodocha (fig. 13), to enclose what has been termed the bulbus lacuna, figs 3, 10 and 13.

Considering each of these in turn.

(i) Receptaculum seminis

Early workers referred to the sperm-storing organ of the bulbus as the vesicula seminis. With Comstock's (1910), Gering's (1953) and Osterloh's (1922) papers this terminology became outdated, and the term receptaculum seminis is now widely used. The term vesicula seminis actually refers to a part of the male genital organs, and receptaculum seminis is a female organ. There is therefore a need for a new name for the sperm-storing organ of the

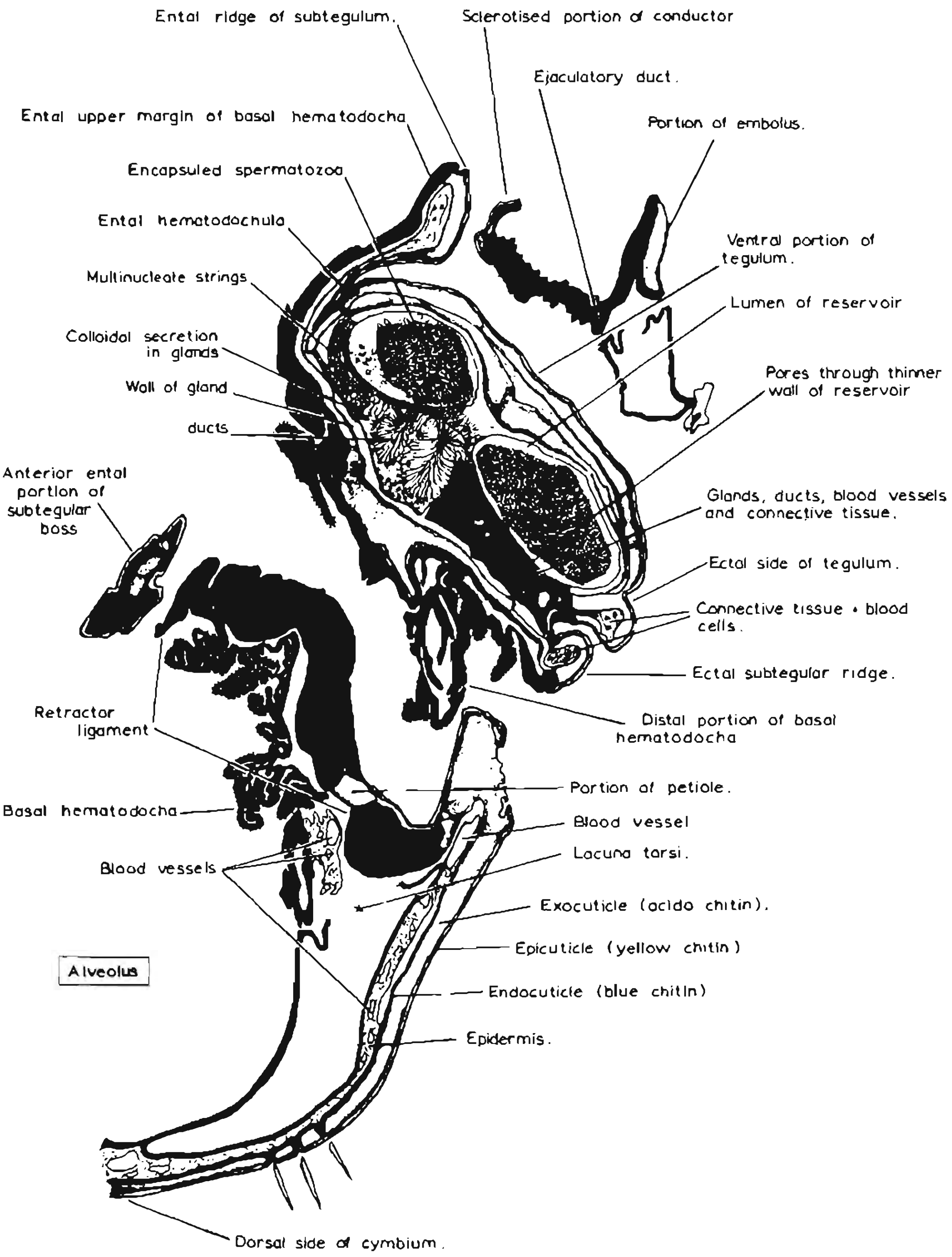
Ental ridge of subtegulum.
Sclerotised portion of conductor
Ejaculatory duct.
Portion of embolus.
Ental upper margin of basal hematodocha
Encapsuled spermatozoa
Ental hematodochula
Ventral portion of tegulum.
Multinucleate strings
Lumen of reservoir
Colloidal secretion in glands
Pores through thinner wall of reservoir
Wall of gland
ducts
Anterior ental portion of subtegular boss
Glands, ducts, blood vessels and connective tissue.
Ectal side of tegulum.
Connective tissue + blood cells.
Ectal subtegular ridge.
Retractor ligament
Distal portion of basal hematodocha
Portion of petiole.
Basal hematodocha
Blood vessel
Lacuna tarsi.
Blood vessels
Exocuticle (acido chitin).
Epicuticle (yellow chitin)
Alveolus
Endocuticle (blue chitin)
Epidermis.
Dorsal side of cymbium.

Fig. 9. Transverse section through right tarsal organ of *P. castaneus*. Frontal aspect of section through X–Y of figs 1 and 4. Complete bulbus and only ectal half of cymbium shown. Bulbus pushed to right-hand side by sectioning, it should come more to left-hand side of page.

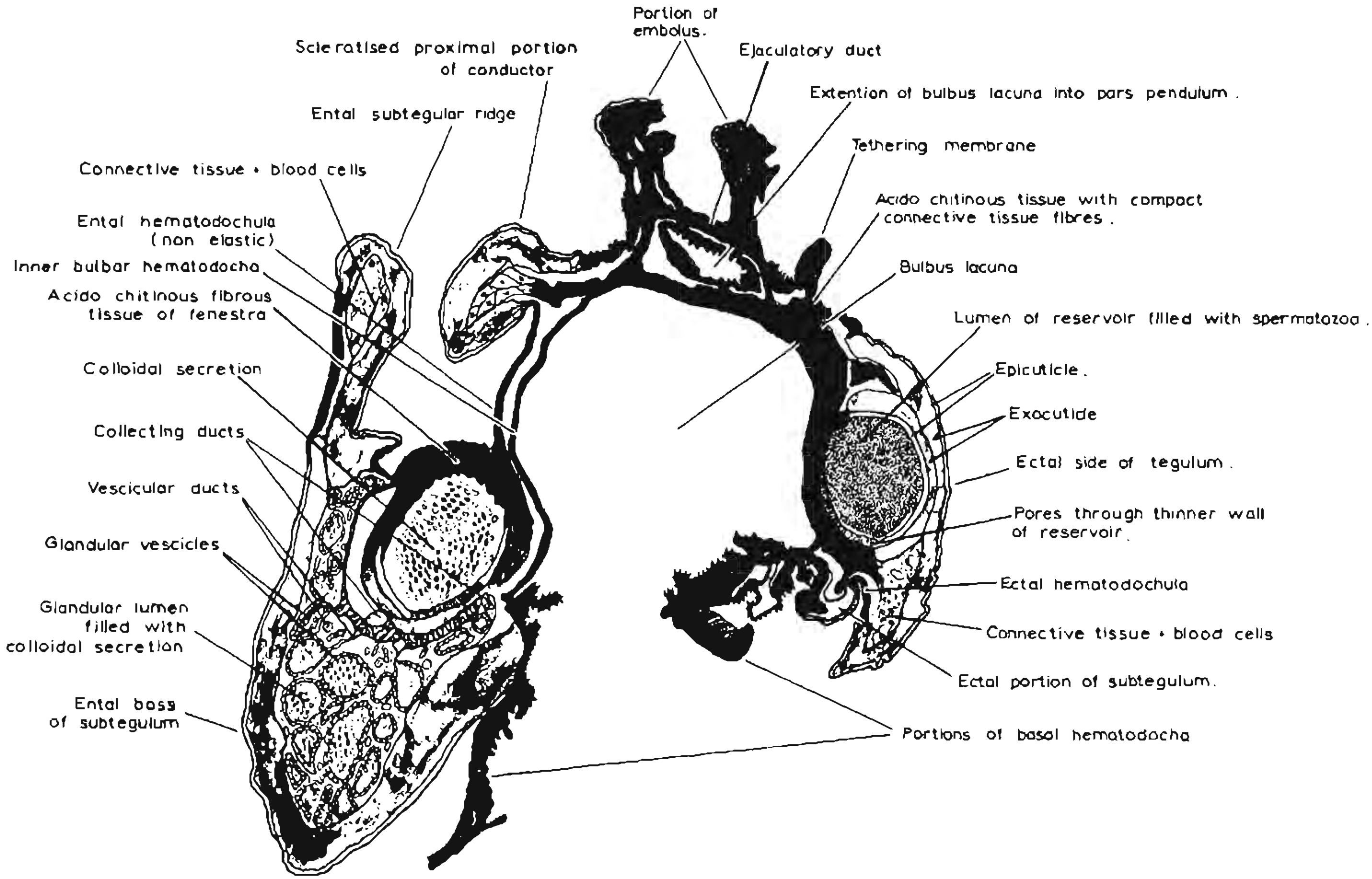
Portion of embolus.
Ejaculatory duct
Scleratised proximal portion of conductor
Extention of bulbus lacuna into pars pendulum.
Ental subtegular ridge
Tethering membrane
Connective tissue + blood cells
Acido chitinous tissue with compact connective tissue fibres.
Ental hematodochula (non elastic)
Inner bulbar hematodocha
Bulbus lacuna
Acido chitinous fibrous tissue of fenestra
Lumen of reservoir filled with spermatozoa.
Epicuticle.
Colloidal secretion
Exocuticle
Collecting ducts
Ectal side of tegulum.
Vescicular ducts
Pores through thinner wall of reservoir.
Glandular vescicles
Ectal hematodochula
Glandular lumen filled with colloidal secretion
Connective tissue + blood cells
Ectal portion of subtegulum.
Ental boss of subtegulum
Portions of basal hematodocha

Fig. 10. Transverse section through bulbus of right tarsal organ of *P. castaneus*. Frontal aspect through region slightly posterior to X_1–Y_1 of fig. 4. Cymbium and hematodocha omitted. Vesicular glands represented semi-diagrammatically but rest of drawing is non-diagrammatic. See fig. 11 for highly magnified drawing of gland.

bulbus in spiders as it is not homologous with either of the above two terms. To avoid confusion the term receptaculum seminis will be preserved in this paper.

The receptaculum seminis of *P. castaneus* and of most spiders consists of a tube ending blindly at its proximal end and tapering considerably to a very small regular diameter at its distal end. It is subdivided into three main regions, namely the ejaculatory duct, the reservoir and the fundus, which is the blind ending. Portions of the receptaculum seminis are shown in figs 1 and 2 by transparency, in figs 3 and 4 as they appear in dissections, and in fig. 6 the whole organ is represented semi-diagrammatically. The entire receptaculum seminis is sclerotized and thus hard, except for a small region on the ventral wall between the distal end of the fundus and the proximal part of the reservoir. I have termed this the *fenestra*, see figs 4, 6 and 10. The fenestra is a tough, non-stretchable membrane that will yield slightly when prodded with a dissecting needle.

The cuticle of the receptaculum seminis of *P. castaneus* was found to be composed of:

(*a*) a layer of epicuticle around its lumen.

(*b*) an incomplete layer of exocuticle along the length of the reservoir and the fundus. The exocuticle is usually well sclerotized.

In transverse section (fig. 9 and ectal region of fig. 10) the exocuticle forms a U-shaped layer around the reservoir. In the region where the fenestra occurs the exocuticle is present only on the ental and ectal walls of the duct (fig. 10—ental side of bulbus). On the distal end of the fundus the U-shaped layer is present again and the exocuticle covers the whole of the proximal end of the fundus. There is thus a band of epicuticle, running along the length of the reservoir and the distal end of the fundus, that is not underlain by exocuticle. I have termed this band the *thin wall.* As it was found to have numerous tiny pores through it, it is also referred to as the thin porous wall (figs 3, 4, 6, 9, 10 and 11). Figure 6 shows that the thin porous wall starts dorsally in the fundus, carries on dorsally in the proximal portion of the reservoir, then migrates towards the inner portion of the reservoir to end at the distal end of the reservoir. The function of the pores in the thin wall will be discussed later.

(ii) The spongy tissue mass

This is the first thing seen upon removal of the tectal plate of the bulbus. Parts of this tissue will tend to be torn away with the tectal plate in fresh specimens but will remain intact in preserved ones. The term spongy tissue is a general one. It refers to all non-cuticular and non-hematodochal tissues found between the inner cuticular walls of the bulbus and the inner bulbar hematodocha. Its histological appearance will be described briefly later.

From dissections the spongy tissue was observed to be composed of two main regions:

(*a*) Projections of fairly tough, tightly woven, non-elastic tissue.

(*b*) Fairly fragile, easily-torn regions composed of glands, loose connective tissue and small blood vessels.

The distribution of these two types of tissues varies a lot within the bulbus, but as a rule it was found that: region (*a*) occurs mainly below the tethering membrane, in parts of the embolus and the median apophysis, and certain regions against the inner bulbar hematodocha. It does not seem, as a rule, to be present near or against the inner cuticular walls of the bulbus (see figs 10 and 13—acido chitinous fibrous tissue). Region (*b*) occurs mainly on the periphery of the tegulum and the subtegulum and opposite portions of the thinner

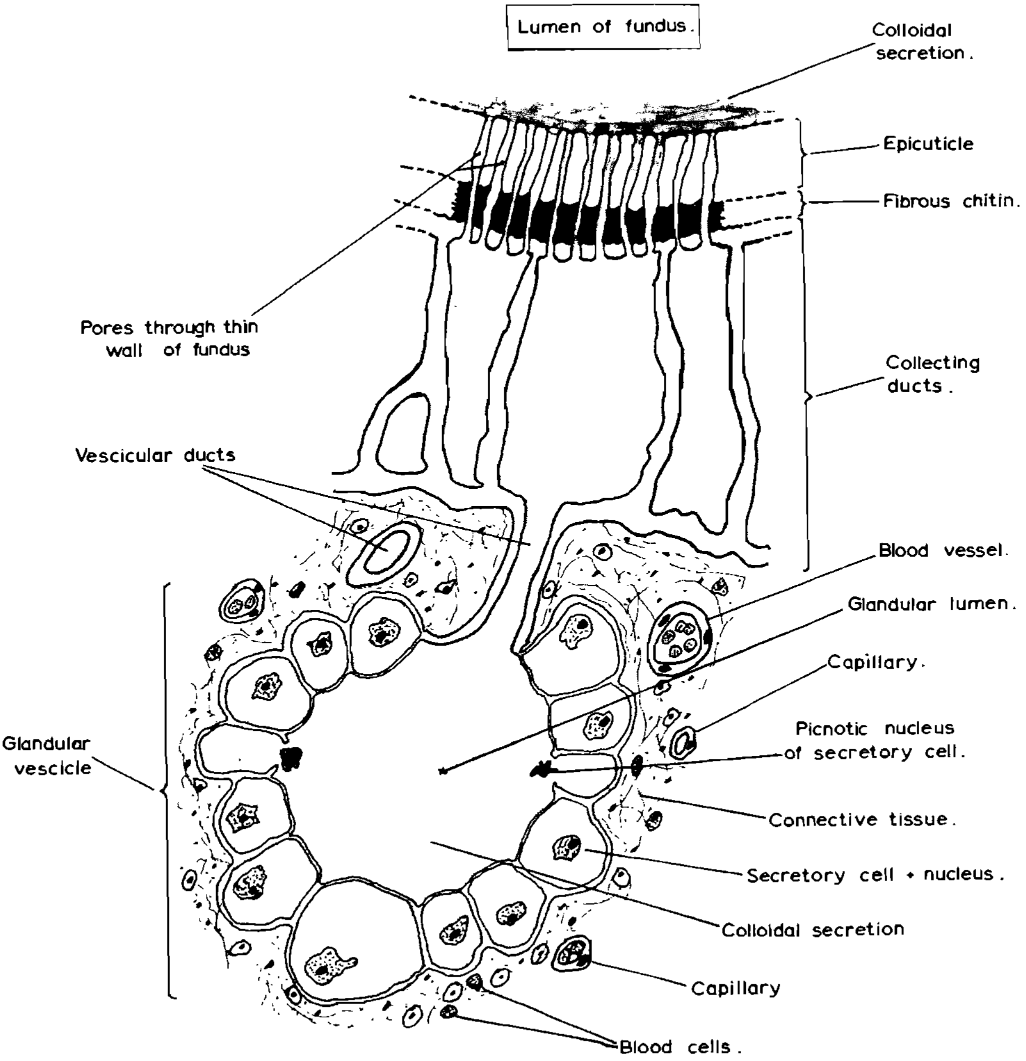

Fig. 11. High-power drawing of a vesicular gland and portion of thin wall of fundus to show pores, collecting ducts, vesicular ducts, a glandular vesicle and small blood vessels.

porous wall of the receptaculum seminis that do not face towards the inner tegular membrane (see figs 9, 10 and 13).

In fig. 3, most of the glandular and loose connective tissue region of the spongy tissue has been removed, to leave mainly the fairly tough non-elastic tissue and the inner bulbar hematodocha surrounding the bulbus lacuna. This portion is seen to have many folds surrounding secondary bulbar lacunae. The spongy tissue also migrates laterally towards the inner margin of the ental subtegular boss (see fig. 2) and the 'condyle' (fig. 4) of the subtegular basal plate and then enters the retractor ligament. Also see fig. 13.

(iii) The inner bulbar hematodocha and the bulbus lacuna

(*a*) *The inner bulbar hematodocha:* This is firmly attached against the inner portion of the spongy tissue and tends to become detached when the spongy tissue is removed from the bulbus. That is why no proximal portions of the inner bulbar hematodocha can be seen in fig. 4. The point of attachment of the inner bulbar hematodocha to the distal end of the basal hematodocha is better seen in figs 10 and 13. The inner basal hematodocha completely encloses the bulbus lacuna and thus is a 'cul-de-sac' for the haemolymph that penetrates the basal hematodocha in the expansion of the bulb. See figs 10 and 13.

The inner bulbar hematodocha has blind ramifications into the pars pendula of the embolus and the proximal part of the median apophysis, thus extending the bulbus lacuna to these regions.

(*b*) *The bulbus lacuna* is a self-explanatory term and has already been referred to above. It is merely a cavity that becomes filled with haemolymph during expansion of the genital bulb.

(2) *The basal hematodocha*

Internally not much more is seen than has already been described. This is basically a membranous tube that is folded spirally upon itself at rest and connects the lacuna tarsi (figs 5, 9 and 13) to the bulbus lacuna. The region of connection between these two, at the proximal end of the basal hematodocha, has been termed the 'orificium hematodochae' by Wagner (see fig. 13). A point of importance is that the basal hematodocha is shorter in the region of the retractor ligament. It cannot thus unfold itself very much when the rest of the hematodocha does. As one moves away on either side of the retractor ligament, the basal hematodocha increases its length and thus its surface progressively to either side of the retractor ligament. In the region of the retractor ligament, the basal hematodocha, as such, faces inwards. As seen above, a portion of the spongy tissue of the bulbus immigrates into the retractor ligament between the retractor ligament with petiole and the basal hematodocha. This region has been termed the *umbilical tissue* for reasons that will become apparent later. The basal hematodocha of that region is thus not in contact with the alveolus (see fig. 13). Progressively away from the retractor ligament on both sides, the basal hematodocha fuses with the retractor ligament and the edges of the petiole. After this it is again in contact with the alveolus space. We see from fig. 13 that there is a blood vessel together with smaller branches travelling through the umbilical tissue of the retractor ligament. The reason for using the term umbilical tissue now becomes apparent, as this tissue provides vascular connection between the bulbus and the secondary tarsal blood vessels.

(3) *The cymbium*

It will now become apparent that the cymbium is mainly the foundation of the tarsal organ. Its anatomy is relatively simple. The retractor ligament is attached to its inner ectal margin (fig. 9). The cymbium has a large blood vessel which enters its proximal end and then swings to the ectal side of the cymbium (fig. 13). This blood vessel (= main tarsal vessel of fig. 13) terminates in the lacuna tarsi and thus supplies the bulbus lacuna, via the orificium hematodochae, with haemolymph. The main tarsal vessel has many secondary branches that move anteriorly (mainly on the ectal and some on the ental side of the cymbium) to the distal end of the cymbium. Some of these go into the umbilical tissue as seen.

The cymbium also has a fairly large nerve that goes only to its distal end. No branches of this nerve were found going to the bulbus via the umbilical tissue. The tarsal nerve mainly supplies the setae and the glands of the distal portion of the cymbium. The cymbium has numerous glands of unknown function in its distal end.

4. *Histology of the tissues of the tarsal organ*

(1) *The cuticle*

Osterloh (1922) showed that the cuticle of the tarsal organ is made up of three layers of chitin identifiable by colour staining properties. He described them as follows:

(i) Yellow-coloured chitin—does not stain. It is hard and is found where strengthening is needed.
(ii) Acido chitin—stained red by eosin. It is found where normal pressure is expected.
(iii) Blue chitin—stained blue by haematoxylin—is found in thin membranous bindings and connecting tubes. This type of chitin is not elastic but is foldable. Blue chitin in the hematodocha becomes thicker in regions and has elastic components.

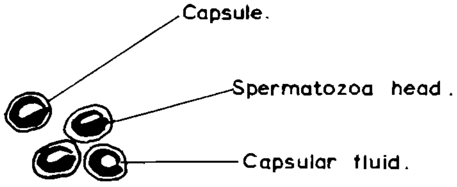

Fig. 12. Encapsuled spermatoza as seen in receptaculum seminis of *P. castaneus*. Nomenclature after Warren 1928.

Basically, Osterloh's observations were confirmed and his nomenclature used, with a few reservations. Yellow chitin was found to be always sclerotized. Acido chitin stained red or purple to mauve, the former colour showing sclerotization and the latter two not. Non-sclerotized acido chitin was found to form the components of fibrous and thick connective tissues, as in the tough region of the spongy tissue and where acido chitinous fibrous tissue occurs in fig. 13. Blue chitin was never found to be sclerotized.

(2) *The bulbus*

The general appearance and the labelling of the various tissues is presented in figs 9, 10 and 13. No detailed account of all the tissues will be presented as this would be too lengthy. A detailed description will be presented only where the histology may be of importance with respect to functional aspects.

(i) The receptaculum seminis

The nature of its walls has been described. Figure 12 shows the appearance of the spermatozoa. It is rather interesting to note that each spermatozoon is encapsulated. A spermatozoon consists of a head and tail, and it is bathed in capsular fluid, presumably a nutrient. Osterloh (1922), Gering (1953) and various other authors have described the presence of taenidia in the lumen of the reservoir of the spiders they investigated. No

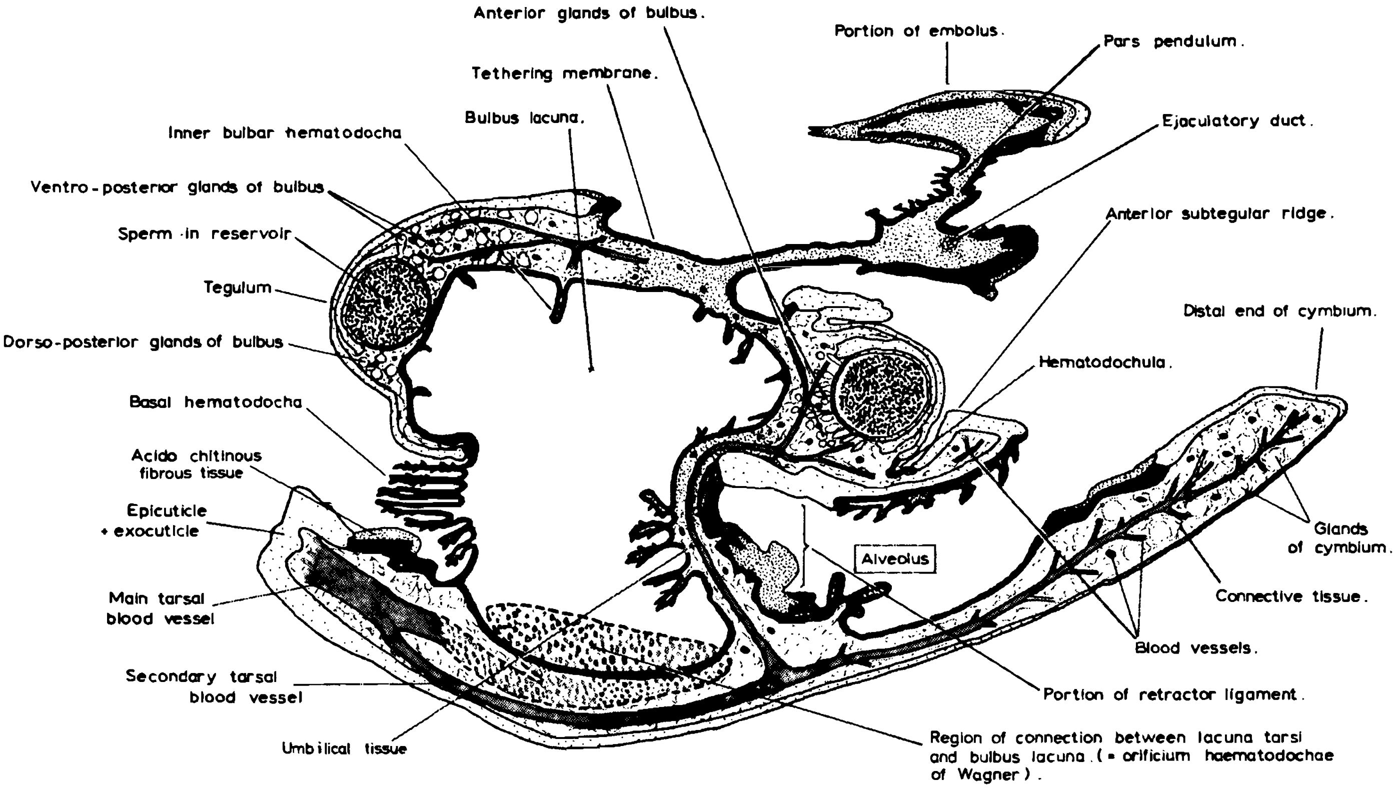

Fig. 13. Semi-diagrammatic representation of a longitudinal section through the right cymbium and bulbus of *P. castaneus* viewed from ental side. Section cut more towards ectal region of tarsal organ.

taenidia were observed in the reservoir of *P. castaneus*. The pores in the thin wall of the fundus are shown in fig. 11. Richards (1953*b*) described pore canals in the cuticle of most insects. These were also found to occur in the cuticle of the cymbium. These pores are the outlets for various secretions on to the cuticular surface. However, the pores of the thin-walled epicuticle of the reservoir and distal portion of the fundus are larger in diameter. There are also some pores in the epicuticular portion covered by the exocuticle of the reservoir and fundus, but these are smaller in diameter. These smaller pores were not drawn as they do not continue through the exocuticle.

By reconstruction from all longitudinal and transverse sections cut, the spermatozoa mass was found to be tightly packed from the distal end of the ejaculatory duct to about halfway between points Y and Y_1 of fig. 4 in the reservoir. The remaining portion of the lumen in the proximal portion of the reservoir and in the bulbus were found to be filled with a secretion. This secretion has a colloidal appearance and has been termed the colloidal secretion. This term was also used by Osterloh (1922).

(ii) Glandular tissue

Two apparently different types of glands were found to occur. These have been termed: (*a*) the vesicular glands, (*b*) the syncytial glands.

(*a*) *The vesicular glands:* These glands occur as vesicles which are lined internally by a single layer of secretory cells (see fig. 11). The secretion, which stains a pale blue colour with Mallory's and Mann's stains, is poured into a glandular lumen and then carried away into a vesicular duct. The length of this duct varies from vesicle to vesicle. The vesicular ducts then discharge their contents into collecting ducts which are thus supplied by several vesicular ducts. The collecting ducts then lead into the lumen of the fundus or reservoir through the epicuticular pores already described (see figs 10 and 11). The colloidal secretion observed in the lumen of the fundus stained a slightly darker blue in some of the sections used and appeared to have darker bands intertwining. The reason for this is unknown. It may well be a staining artefact as this phenomenon was only observed in certain sections stained with Mallory's stain and did not appear in the sections stained with Mann's stain. The glandular tissue usually contains many such vesicles. Between the vesicles one observes connective tissue with scattered nuclei and many blood cells. Also visible are many small blood vessels and occasionally some capillaries. The blood vessels also contain many blood cells. No blood vessels were ever seen to lead into the epicuticular pores or into blood sinuses that might lead into the epicuticular pores. No blood cells were ever seen in any collecting ducts near the pores. The epicuticular pores are always separated from any connective tissue containing haemolymph by a membrane or by thick connective tissue. In such instances the pores are then found to be blocked. This is particularly well seen where the pores of the thin epicuticular wall face inwardly towards the bulbus lacuna on the lateral ectal and ental sides of the bulbus. See fig. 10.

The vesicular glands occur in large numbers in the region around the fundus (inside the ental subtegular boss) and the proximal portion of the reservoir. Along the fundus towards its distal end and the proximal end of the reservoir, these glands are found less and less abundantly and eventually are absent where syncytial glands first appear.

(*b*) *The syncytial glands*: Suomalien (1964) worked on the histology of the poison glands of araneids. He showed that the secretory part of the glands forms 'a syncytium consisting of star-shaped cells with very long branches'. Although the region of occurrence of this second type of glandular cell was rather badly damaged in most of the sections, it appears that a similar type of syncytium is present along certain portions of the reservoir only. The best reconstruction that could be obtained is represented in fig. 9. These syncytial glands consist mainly of two parts, i.e. the syncytial cell bodies arranged in the multinucleate strings or along the walls of the glands (fig. 9) and secondly the ducts (fig. 9) which collect the secretion and go directly to the epicuticular pores. There are no collecting ducts. The secretion of these glands also appears to have a colloidal nature but stains more purplish with Mallory's and Mann's stains. This purplish-stained secretion was observed to occur in the glands, ducts and in the lumen of the reservoir wherever there was sperm.

Moving away from the proximal end of the reservoir, these glands start occurring where the vesicular glands end. They are found to be most abundant in the anterior region of the tegulum around the porous thin wall of the reservoir. They also occur to a lesser extent as one moves towards the distal end of the reservoir where they eventually cease. There are many small blood vessels in the connective tissue between these glands. The marked difference in colour displayed between the colloidal secretion of the vesicular glands and the syncytial glands in all slides scrutinized strongly suggests a different type of secretion. This is well marked where the proximal end of the sperm mass is in contact with the distal end of the vesicular gland secretion in the lumen of the proximal region of the reservoir. The change in colour from purple to pale blue is sharp.

(3) *The hematodocha*

(i) The basal hematodocha

Histological and anatomical investigations of the basal hematodocha yielded interesting new findings. We have seen that the hematodocha, when expanding, becomes untwisted and unfolded at the same time. This movement is conveyed to the bulbus, which swivels to the ectal side of the cymbium. I became interested in what structural factors might cause this and found that the hematodocha is composed of:

(*a*) *non-elastic chitin fibres* (as judged from staining reactions), forming strands of varying thickness and surface. These can be subdivided into:

— Fibres forming strands of varying thickness that run into a helical or spiral path from the proximal end of the hematodocha (in cymbium) to the distal end of the hematodocha attached to the dorsolateral periphery of the bulbus. These have been termed the primary strands of the hematodocha (see figs 14 and 15). They run in a clockwise direction when viewing the right cymbium ventrally as in fig. 5.

— Fibres forming strands that are thinner, shorter and interconnect the primary strands. These have been termed the secondary interconnecting strands (figs 14 and 15).

(*b*) *the elastic chitin fibres*: These fibres are elastic (as shown by use of Orcein and Van Gieson's stain) and interconnected with each other, forming an intertwining sheet between the frames formed by the primary and secondary non-elastic strands (see fig. 14). They have been termed elastic chitin fibres as they also stain blue with Mann's stain, thus showing

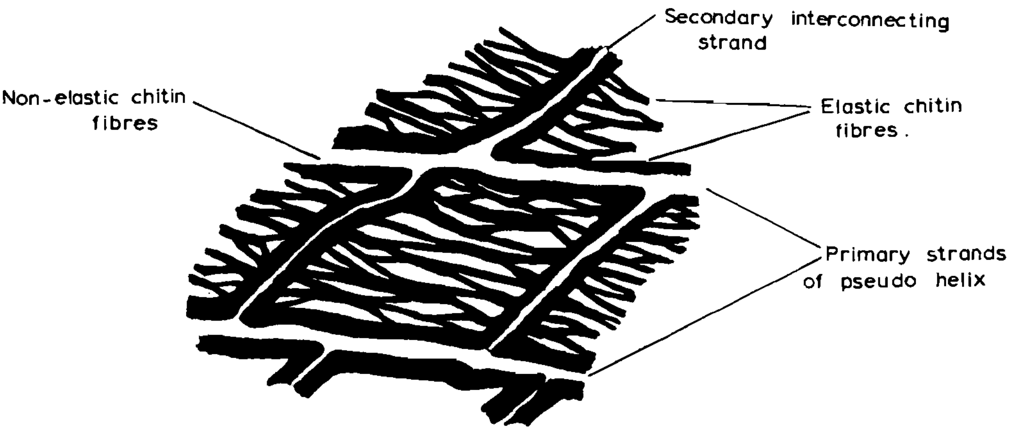
Secondary interconnecting strand
Non-elastic chitin fibres
Elastic chitin fibres.
Primary strands of pseudo helix

Fig. 14. Semi-diagrammatic drawing of hematodochal fibrous tissue to show various components.

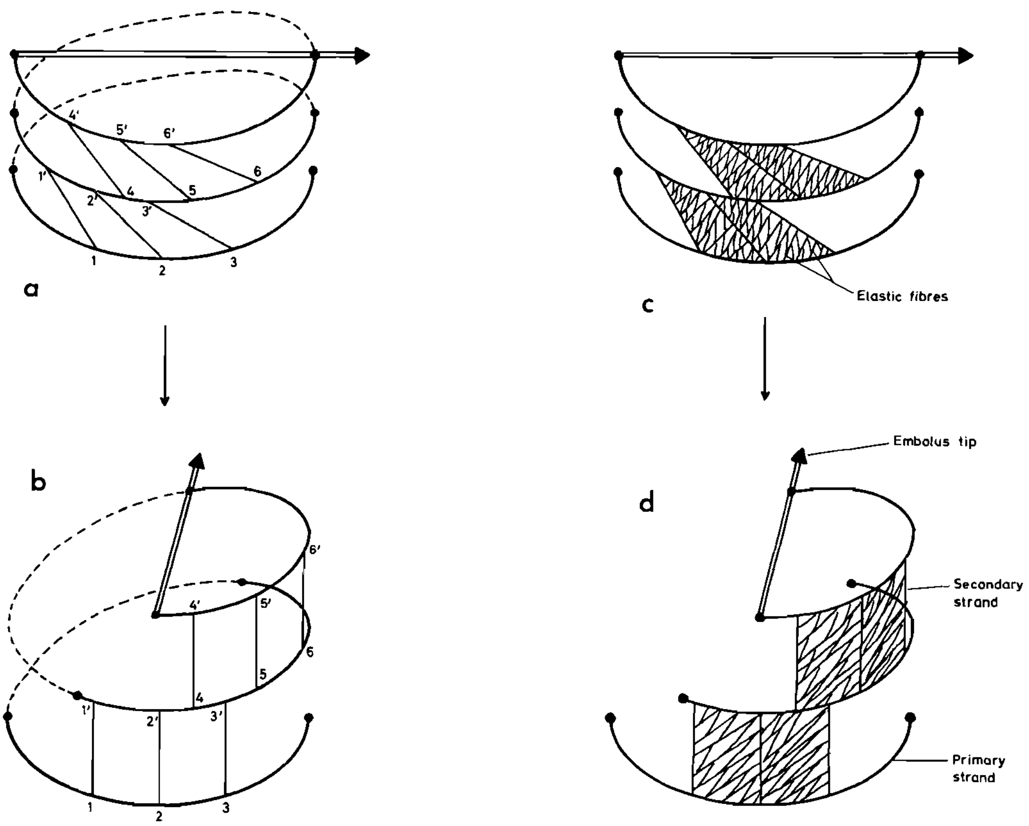

Fig. 15. Diagrammatic three dimensional representation viewed from the ental side to show how fibrous and elastic components of the hematodocha move when the bulbus is pushed out by raised haemolymphatic pressure. Only portions of primary strands are shown to avoid overloading of the drawings. The 'embolus tip' (arrow head) is swivelled in an anticlockwise direction in *b* and *d* when the bulbus is pushed out. In *a* and *b* only the primary and secondary strands are shown. In *c* and *d* the pseudo-helical path of the primary strand (broken line) has been omitted, but the elastic fibres are shown. *a* and *c* show resting while *b* and *d* show active states. See text for further explanations.

the presence of blue chitin mixed with the elastic fibres. With the Orcein and Van Gieson stain, small patches of negative areas for elastic tissue were found amongst these fibres, further explaining the above terminology. The function of blue chitin is probably to occupy the spaces between the elastic fibres when the hematodocha is completely expanded. The most likely function of these various parts will be presented in the discussion.

(ii) The inner bulbar hematodocha

This is fairly similar to the basal hematodocha, but there are less elastic fibres and more non-elastic chitinous fibres. Folding also occurs to a limited extent. It could not be established whether the pseudohelix of the basal hematodocha (to be discussed later) continues into this portion.

(iii) The hematodochula

This is tougher than the basal hematodocha and does not contain any elastic fibres. It is thus not resilient.

5. *Experimental investigations*

(1) *Attempted mechanical ejaculation*

Most authors consulted, except Osterloh (1922) and Harm (1934), have suggested, but not proved, that ejaculation of the sperm is brought about by the raised hydrostatic pressure of the haemolymph either squeezing the bulbus or causing the collapse of the whole receptaculum seminis. While it is not suggested that this is never the case for other spiders, I maintain that this cannot occur with *P. castaneus* due to the complete sclerotization of the receptaculum seminis (except for the fenestra).

To confirm this the following simple experiment was performed. The palpus of an anaesthetized adult male of *P. castaneus* was cut off at its proximal end. As no references to an arachnid Ringer solution could be found, an insect Ringer solution, as described for *Apis* larvae by Pantin (1964: 67), was prepared. This solution was injected slowly into the palpus prepared and arranged as explained for other injection experiments, and the process of expansion observed under the high power of a dissecting microscope. The pressure was increased until the hematodocha eventually burst. At no stage could any seminal fluid be seen to come out of the embolus tip. The receptaculum seminis together with a portion of the bulbus was then dissected out of the bulbus, desclerotized (*not* 10% KOH but diaphanol) and cleared. A column of sperm was observed in the lumen. Samples from various regions of the lumen were then withdrawn, spread on a slide and observed under a compound microscope. All samples, except in the fundus region, were found to contain sperm.

(2) *Effect of hinging action of tegulum at the hinge region*

It was thought that the hinge action described earlier in the section dealing with the expansion of the genital bulb might cause a depression of the fenestra. This hinging action was simulated by pulling on the ectal side of the regulum (the tectal plate and spongy tissue having been removed) and keeping the basal hematodocha down. The ectal side of the tegulum was found to move slightly away, but only as far as the ectal hematodochula would allow it. No marked depression of the fenestra was caused by the hinging movement. Furthermore this movement did not cause any distortion of the receptaculum seminis and

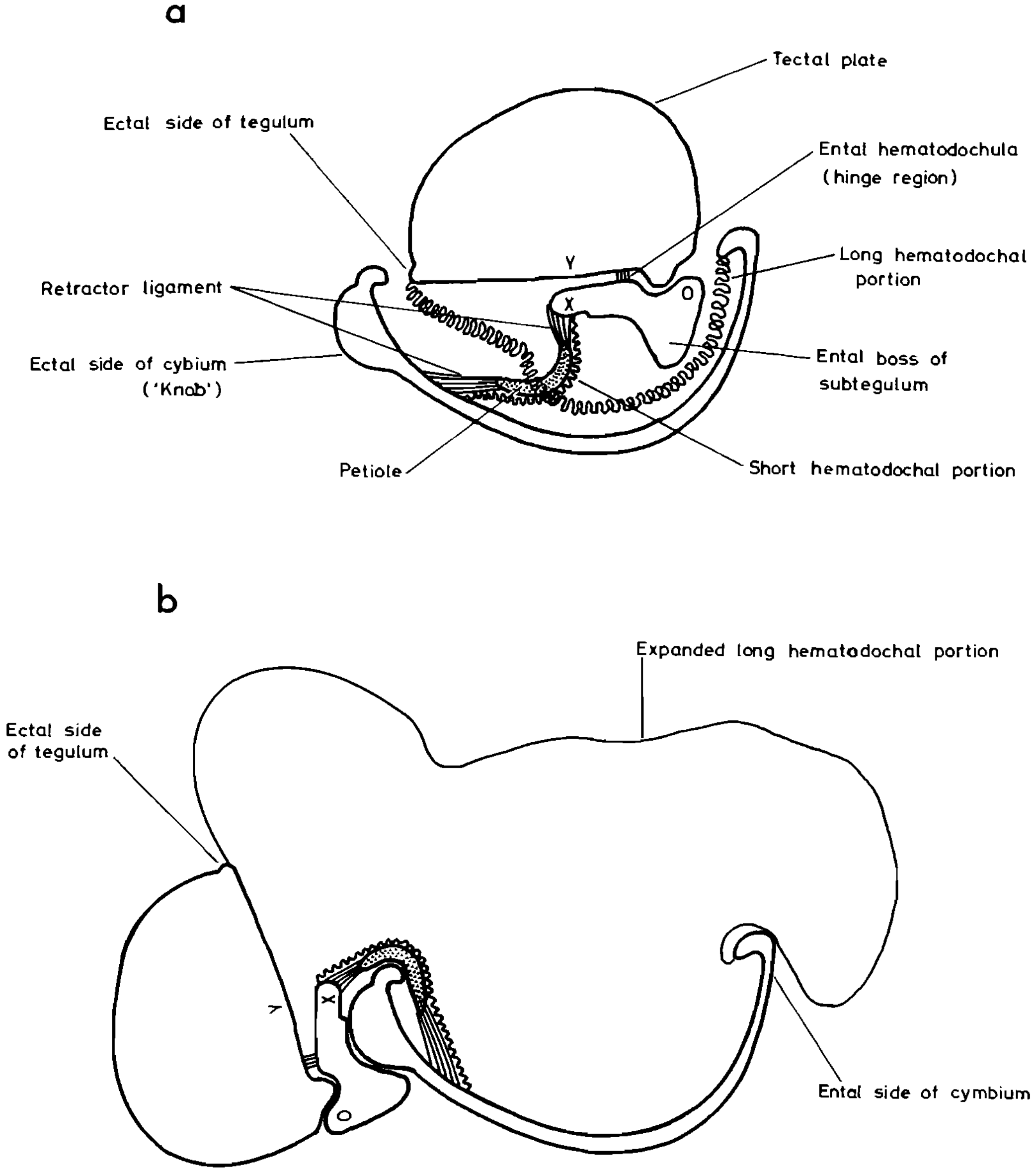

Fig. 16. Diagrammatic representation of transverse sections through the right tarsal organ of *P. castaneus* in the region of the retractor ligament, petiole, tegulum and subtegular boss. Section viewed along the antero-posterior axis of the cymbium from the proximal end. *a*, position of the various parts at rest. *b*, position of the various parts after the bulbus has swivelled round to the ectal side of the cymbium due to expansion of the basal hematodocha. X, Y and O are symbols to help relate the various parts to each other. The retractor ligament is not completely drawn. It should also run below the petiole and have portions of its proximal part also attached to the inner wall of the 'knob' of the cymbium. See text for further explanations.

no sperm was seen ejaculating from the embolus tip. This confirms that mechanical distortion cannot cause ejaculation in *P. castaneus*.

(3) *Effect of capillary action on sperm induction*

Most authors who considered the problem of sperm induction suggested, but did not prove, that the small size of an empty receptaculum seminis lumen produces a strong capillary action which draws the seminal fluid from the sperm web into the ejaculatory duct. Kaston (1948) stated that 'it is possible that sperm induction is effected by a lowering of the pressure around the sperm duct, causing the semen to be sucked in'. The following experiment was performed under a dissecting microscope. A complete, undamaged receptaculum seminis was removed from a tarsal organ that had been desclerotized by boiling in 10% KOH. The proximal end of the fundus was cut off and all remaining luminar content of the receptaculum squeezed and sucked out. (No damage was done as it was desclerotized.) This was then dried by gently blowing on it and allowing it to stand for a while. The distal end of the fundus was then plugged with a droplet of glue, and the whole surface of the receptaculum varnished with thinned, quick-drying, transparent nail varnish and allowed to dry. After a few minutes the embolus tip was dipped in a droplet of ordinary blue fountain-pen ink. The ink was seen to rise to just over 80% of the length of the reservoir and fundus. This level is just about in the vicinity of the proximal end of the reservoir. The same experiment was repeated without varnishing the receptaculum, and this time the ink filled the whole structure.

VI. DISCUSSION AND CONCLUSIONS

1. *Sperm induction mechanism*

We saw that capillary action had been suggested as the mechanism operating such induction, but had never been proved. The results obtained in the experimental section of this paper seem to support this hypothesis. Why did the level of ink rise further in the unvarnished receptaculum seminis? This is probably due to the fact that the air trapped in the bulbus can escape through the epicuticular pores (of the unvarnished vesicula seminis) and will thus not produce a counter-pressure which would counteract capillary pressure as experienced with the varnished receptaculum. What happens in living spiders? How is this counteracting air pocket dealt with? Only hypothetical answers are possible. Is there in fact only air present in the vesicula seminis or is there a fluid that is withdrawn by the tissues around the vesicula seminis, through the epicuticular pores, during sperm induction? This could only be answered by investigating the tarsal organs of young males after the penultimate moult and just prior to sperm induction. If only air is present and no secretions, is this counteracting air pocket slowly absorbed or does it remain? It is possible to answer the latter part of this question from certain observations already presented. The remaining portion of the lumen in the proximal end of the reservoir and in the fundus is filled with a colloidal secretion. This secretion is in contact with the proximal end of the sperm mass in the receptaculum seminis. There is thus no air pocket between the two. This would suggest two things:

(*a*) if only air was present initially, it was absorbed. How? This is unanswerable, but it may be suggested that as carbon dioxide (0,8% of air) is very soluble, this could slowly diffuse through the pores and then into the haemolymph (through the glandular walls) of the spongy tissue. Oxygen (21% of air) could also be diffused through in the same manner, especially if the oxygen tension in the haemolymph is low. But how is nitrogen (78% of air) got rid of? If the nitrogen tension of the haemolymph is low, then a similar process of diffusion may occur.

(*b*) No air was ever present and the receptaculum seminis contained a secretion that is absorbed as the sperm is forced into it by capillary action. There would be no counteracting haemolymphatic pressure against this process as most authors report that the genital bulb is not expanded during sperm induction.

Gering (1953) observed that: 'The palpus is raised from time to time permitting the fluid to flow down through the reservoir into the fundus.' But he did not consider whether the receptaculum seminis has air or not in it prior to induction.

Another factor to be considered is that the receptaculum seminis is an invagination of the cuticle as Harm (1934) and Barrows (1925) showed in their reports on the study of the development stages of the tarsal organ. They do not say whether air or a secretion is present in the receptaculum seminis prior to sperm induction. This would perhaps suggest that only air is present at the preinductory stage.

To sum up, the results obtained in this investigation definitely show that capillary action could be the mechanism operating sperm induction. Cooke (1966) states: 'The most plausible hypothesis is that the lumen of the reservoir is filled by secretions from the palpal glands and that seminal fluid is drawn up by resorption of these secretions.' However, this is pure conjecture and has not yet been verified experimentally.

2. *Sperm storage*

It was found that in most cases the sperm is found in the ejaculatory duct and 80% of the reservoir and fundus length. Is this the maximum amount of sperm that can be stored? This cannot be answered. Osterloh (1922) reported that the sperm filled the whole of the vesicula seminis in all the specimens he studied after sperm induction. The fact that the reservoir and fundus contained sperm in only 80% of their combined length in the case of *P. castaneus* may be due to the fact that the specimens caught had already mated and thus only a certain amount was left for further matings. If this is correct, it would imply that the spider does not empty its receptaculum seminis completely in the course of a single mating.

3. *Sperm ejaculation mechanism*

Let us first sum up what we can accept with certainty regarding this problem:

(i) The receptaculum seminis is completely sclerotized except for the small fenestra.
(ii) There are no muscles in any part of the bulbus that could squeeze any portion of the receptaculum seminis or cause ejaculation in any other way.
(iii) It was shown earlier by an experiment labelled 'attempted mechanical ejaculation' that the increased hydrostatic pressure, which causes expansion of the genital bulb, does not in any way cause ejaculation.
(iv) It was also shown that the hinging movement of the tegulum on the subtegulum did not cause any depression of the fenestra or appreciable distortion of the receptaculum seminis.

(v) The bulbus lacuna has no connections with the spongy tissue of the bulbus.

(vi) An alternative way for vascular supply to the spongy tissue, that is, through the umbilical tissue, is shown to occur.

(vii) No blood vessels are in contact with the epicuticular pores. No haemolymph or blood cells were found in any cavity that leads to the epicuticular pores.

(viii) The spongy tissue has been shown to be composed of two apparently different types of glands, namely (*a*) vesicular glands, mainly leading into the fundus epicuticular pores, (*b*) syncytial glands, mainly leading into the reservoir through the epicuticular pores.

(ix) The colloidal secretion of the vesicular glands was found in the glandular lumen, their ducts and the proximal portion of the receptaculum seminis. The colloidal secretion of the syncytial glands was found amongst the syncytial nuclei, the ducts and in the lumen of the receptaculum wherever sperm capsules are present.

(x) The staining properties of the colloidal secretion produced by these two types of glands are different, suggesting different types of secretions.

(xi) There is no nerve supply to the bulbus either via the umbilical tissue or any other path.

Let us now briefly review the more popular theories concerning ejaculation. It has to be stressed here that even if some of these theories have to be rejected for this investigation, it does not mean that they are incorrect for other groups or species of spiders.

The various theories encountered in the literature consulted were:

(*a*) The pressure exerted by the haemolymph at maximal expansion squeezes the fundus or causes collapse of the receptaculum seminis, thus forcing the sperm out. These are the views held by Bristowe (1930–58), Berland (1932), Comstock (1910), Simon (1892–5) Gerhardt (1923–33), Gering (1953) and several others. While these authors may be correct for the spiders they investigate (some are definitely not), this theory certainly cannot apply to *P. castaneus* because of statements (i) and (iii) outlined above.

(*b*) Muscles are present around the fundus and squeeze it. This was the earliest of all theories but was discounted by most authors at the end of last century. Bristowe (1929) however still held it then for the Tetrastica (most primitive spiders). Statement (ii) above in any case discounts this for *P. castaneus.*

(*c*) Hinging of the tegulum on the subtegulum at the tegular-subtegular articulation due to raising of the tegulum (thus indirectly by increased haemolymphatic pressure) causes a folding of the fundus (or the fenestra in this investigation). These are the views of Gering (1953) and Comstock (1910) and Osterloh (1922) for certain spiders only. This is contradicted by statements (i) and (iv) for *P. castaneus.*

(*d*) The lumen of the receptaculum seminis communicates with the haemolymphatic vascular system through the epicuticular pores. When the haemolymphatic pressure rises, the haemolymph penetrates through the pores pushing the sperm out. This was the view held by Bertkaw (1884) and Wagner (1887). It is contradicted by statement (vii) in the case of *P. castaneus.*

(*e*) Sudden release of colloidal secretion by the spongy tissue glands, through the epicuticular pores, into the fundus lumen causes the sperm to be driven out (Szombathy 1915, Osterloh 1922 and Harm 1934). Osterloh (1922) furnishes as evidence for his theory an amazing histological difference between the glands of males that have just copulated and those that have not. He further suggests in his paper that blood pressure, especially when high, stimulates the glands to secrete. This theory, with a few reservations and additional observations made by myself, seems to be correct for *P. castaneus* in view of statements (viii) and (ix). The probability of this being the correct interpretation is further increased by the fact that there is no factor, from observations gathered in this investigation, that points to an alternative mechanism.

The results obtained in this investigation also imply that the vesicular glands, because because of their structure (i.e. they could release all their secretion at once into the vesicular lumen and thus into the fundus lumen) and their position (near the fundus mainly), produce the secretion that actually pushes the sperm out of the vesicula seminis. However, it is rather doubtful that the glands themselves actually provide the pressure (caused by the sudden release of colloidal secretion) to push the sperm mass out of the vesicula seminis. This is the point of view held by Osterloh (1922); he postulated that it is the collapse of the glandular walls of the vesicles that drives the colloidal secretion into the fundus lumen. To substantiate his hypothesis he draws a comparison with the prostate gland of mammals, which produces a secretion that presses the sperm out. Although the prostate in mammals plays a role at the moment of ejaculation, ejaculation is not caused by it but by muscular contraction. His analogy is thus invalid. I know of only one instance where sudden release of a secretion might actually exert enough pressure to cause movement of a structure. This apparently occurs in the eye accommodation process of the Polychaete *Vanadis*. The resting eye is said to be focused on infinity. To focus on a nearer object, the lens is pushed away from the retina by the rapid secretion of a fluid gel from glands under the eye. But this mechanism has never been proved actually to work and was deduced from anatomical and histological investigations.

I would rather think that at the critical moment, that is, when all copulatory requirements such as locking etc are fulfilled, there is a mechanism that causes all or only a portion of the secretory cells in the vesicular glands to release their secretion in the vesicular lumen. As the cell walls break into the glandular lumen and do not offer further turgid resistance to the high haemolymphatic pressure then acting in the bulbus lacuna and on the spongy tissue as a whole, this pressure can then squeeze the colloidal secretion into the vesicular ducts, the collecting ducts and then the fundus lumen, via the epicuticular pores, to push the sperm out. In this event, the hydrostatic pressure of the haemolymph would then indirectly by means of the liberated colloidal secretion mass, cause sperm ejaculation. This would seem more feasible than Osterloh's hypothesis. Another possibility is that the sperm is pushed out by a combination of the two forces, i.e. haemolymphatic pressure on the glands and the released turgescent pressure of the glands themselves.

The structure of the cells in the vesicular glands (see fig. 11) would suggest an eccrine or apocrine type of secretion and it is most probable that the former is the correct one as this would imply a faster rate of secretion. One may argue that the picnotic and liberated nuclei in the glandular lumen in fig. 11 suggests a holocrine type of secretion. This is rather doubtful as holocrine secretion in other organisms is usually a slow process. The answer to this question would really be to cut sections of the tarsal organs of a male that has just mated.

What is the mechanism, if any, that triggers the release of the secretion? Without a triggering mechanism the glands could release their secretion at any time. How could the spider ensure, for instance, that the sperm is not ejaculated before the embolus is well inside the bursa copulatrix of the female? A triggering mechanism must be present. Let us consider the possible nature of such a mechanism.

— Because of statement (xi) above, nervous stimulation of the glands is not considered possible.

— Osterloh's only suggestion is that blood pressure, especially when high, stimulates the glands to secrete. This seems possible, especially if we refer back to Gering's (1953: 16) description of mating. He observed maximal expansion just prior to and at the time of ejaculation, together with a rhythmic slight increase and decrease of pressure at that time, as witnessed by maximal raising and slight lowering of the spines on the palpi. It would seem that the slight increase of pressure at maximal expansion is enough to trigger the glands to discharge their secretion. There is, however, one factor that seems to contradict this, namely: that the high pressure exerted during attempted mechanical ejaculation described earlier did not cause the glands to release their secretion and thus cause ejaculation. This experiment seems to invalidate Osterloh's hypothesis.

— There is an alternative, very plausible way in which the secretory glands could be triggered. It appears that the release of a hormone could act as a trigger. Many hormones are known to affect secretory processes both in vertebrates and in invertebrates. This is further substantiated by Cooke's findings (1966: 41) that in *Dysdera*, 'By injecting blood samples drawn from copulating males into non-copulating individuals it was possible to show in a controlled pilot experiment that stimulation of the palpal glands is brought about by an endocrine factor'. Cooke also showed, by drawing samples from various parts of the body, that the hormone appears to be liberated in the distal portion of the palp and that this localization suggests neurosecretory mechanism may be involved.

I have recently repeated Cook's experiments using males of *Palystes natalius*, and obtained similar results, although I was only able to withdraw blood samples of copulating males from pedipalp segments and not the prosoma due to insufficient numbers of specimens. In *P. castaneus* the vascular supply to the spongy tissue and thus the glands is separate, that is through the umbilical tissue. The exact site and the factors initiating the production of this hypothetical hormone are as yet unknown. The site could either be in the distal end of the cymbium or the prosoma. Initiation would presumably involve a nervous stimulus caused by mechanical stimulation of setae or spines in the distal end of the palpus or same type of sensory stimulation. Rhythmic pumping of haemolymph prior to and during ejaculation, mentioned above, would explain how the hormone produced is forwarded to the secretory cells. This would be done by the numerous small blood vessels described near the vesicles. This hypothesis would explain why ejaculation did not occur during the attempted mechanical ejaculation experiment.

One may object that the high haemolymphatic pressures of the bulbus would tend to squash the spongy tissue and thus prevent circulation in them. This is not correct because one must remember that the haemolymph which causes high pressure in the bulbus lacuna also has access to the spongy glands via the umbilical tissue blood vessels. Haemolymphatic pressure would thus be equal on both sides of the inner bulbar hematodocha. There is one difference however: the blood in the bulbus is in a vascular 'cul-de-sac' whereas the blood in the spongy tissue can circulate. Haemolymph would thus be caused to rush into the umbilical tissue blood vessels with each rhythmic rise in pressure witnessed by Gering (1953) (and other authors as well) at maximal expansion, and at the same time blood would be forced back through the connective tissue of this umbilical tissue (similar to venous return in closed blood systems).

One may further object that the twisting of the retractor ligament caused by the swivelling movement of the bulbus may close off the blood vessels of the umbilical tissue. This is very unlikely for various reasons. It was noticed that, at rest, the distal end of the retractor ligament is already slightly twisted in a clockwise direction (when viewing the right cymbium ventrally). This is partly seen in fig. 5. During expansion, the anticlockwise swivelling of the bulbus will, if anything, tend to untwist the retractor ligament, thus probably improving circulation by widening the space occupied by the umbilical tissue. The petiole will also protect the region of the retractor ligament that rests on the ectal rim of the cymbium and protect the tissues inside the retractor ligament. Finally, the retractor ligament itself is a fairly rigid structure and any strangulation that might occur will be counteracted.

What is the function of the syncytial glands?

We have seen, in the light of statement (x), that these glands probably secrete a different type of colloidal secretion into the reservoir. No clues as regards their function are available but a few suggestions are offered. As this secretion is mainly found in and around the sperm mass, it is possible that its function is to bathe the encapsuled spermatozoa and keep them moist. The nature of the capsular wall is unknown, but it may be such that it allows nutrients to pass through from this secretion. This secretion may also act as a lubricant and prevent squashing of the capsules during ejaculation. It may also act to keep the sperm mass coherent, and if this secretion is of a completely different nature from that of the vesicular glands, it may form a suitable interphasal front for the pushing mass of the vesicular gland secretion during ejaculation.

It is doubtful whether these glands have any direct function in ejaculation. Being of a syncytial nature, one cannot see how they could be triggered to discharge suddenly a great amount of secretion. Syncytial secretory cells as a rule (e.g. poison glands of spiders) secrete slowly; the secretion is then usually stored in the centre of the gland, and then made use of when required. In the case of the present syncytial glands, this would seem to apply as well. Their secretory product would then be pushed into the reservoir lumen by the raised haemolymphatic pressure and thus perform some of the possible functions outlined above. At rest they might only keep the sperm moist and possibly nourish it.

One may ask whether the syncytial glands do not also secrete an enzyme that dissolves the sperm capsule prior to ejaculation. Female spiders usually possess a spermatheca to store the sperm. Most authors that refer to sperm activation think that this is initiated by the female only. Thus it is very doubtful whether the syncytial glands could activate the spermatozoa.

To summarize what has been discussed so far on ejaculation, it is postulated (speculatively in some stages) that ejaculation in *P. castaneus* is affected in the following manner:

(i) The vesicular glands, when triggered to secrete, provide a secretion that can be released to drive the sperm out. This mechanism could be compared to the piston of a steam-engine.

(ii) The raised haemolymphatic pressure at maximal extension forces the released secretion into the fundus, causing the sperm mass to shift distally. This factor could be compared to the pressure of the steam driving the piston (one way only) in a steam-engine.

(iii) The triggering factor is most probably a hormone released just prior to a maximal expansion of the genital bulb.

(iv) The syncytial glands do not help appreciably in directly driving the sperm out, and probably have secondary functions such as providing a lubricating, nourishing, bathing and cohesive medium.

4. *The mechanism and functions of the basal hematodocha, the retractor ligament and the petiole*

The hematodocha and its components were described above (section on results). In all references consulted the detailed structures of the hematodocha have not been described for any spider and the mechanics of these components are unknown. An interpretation of these results will thus be dealt with.

The whole structure is very much like a fishing-net, forming a cylinder, the squares formed by the twine of the net being the primary and secondary strands of figs 14 and 15. Thicker twine would form the primary strands and run in a helical path along the vertical axis. Elastic fibres positioned as shown in fig. 14 would cause traction on both the primary and secondary strands. If reference is made to fig. 14, this can be seen to be done in two ways:

(i) The main elastic fibres, running between secondary strands and some—especially near the base of a primary strand—running from a secondary to a primary strand, will tend to pull the secondary strands parallel to the primary strands, that is a folding of the secondary strands to the right in fig. 14 and to the left in fig. 15*b* and *d*.

(ii) The shorter elastic fibres joining the main elastic fibres to each other, and also to the primary non-elastic strands, will tend to bring the helical primary fibres towards each other.

Both these situations will cause a swivelling movement of the helix of the primary non-elastic chitin strands in a certain direction (these strands being stiffer and non-stretchable). This swivelling movement will in turn be transmitted to the bulbus as the cymbium cannot swivel round. In fig. 15, assuming that we are looking down on to the ventral side of the tarsal organ, that is looking along the axis of the arrow between *a* and *b*, the bulbus will swivel in an anticlockwise direction.

So far in our considerations we have assumed the presence of only one primary non-elastic strand following a helical path. This is only theoretical, for in actual fact there are many of these primary strands. They are all interconnected by secondary strands and elastic chitin fibres in the fashion described above.

When viewing the right pedipalp ventrally as in fig. 7, we have seen that the embolus tip swivels anticlockwise and follows more or less the path indicated by the heavy broken line on the ectal side of the cymbium. We have also seen that the ental side of the subtegulum (boss region) comes to rest against the ectal side of the cymbium. This is due to the retractor ligament. We saw that the hematodochal membrane is shorter in the region of the retractor ligament. This has been labelled the short hematodochal portion in fig. 16*a*. The portion of the hematodochal membrane that connects the ental side of the alveolus to the lower ectal region of the bulbus (right tarsal organ) is longer and has been termed the long hematodochal portion in fig. 16. Bearing this in mind we have to reconsider the term 'helix' used above when talking about the path of the primary strands. It is obvious that:

(i) the axis of the helix is not straight;

(ii) the long hematodochal portion is caused by the fact that this region will have longer primary non-elastic strands, and accordingly the short hematodochal portion will have shorter primary strands. Thus the helix of the primary strands is a rather distorted one. For this reason I have called the path taken by the primary strands a pseudohelix.

The folding of the basal hematodocha observed in figs 2 and 5 is caused by groups of certain primary strands that are progressively longer away from the retractor ligament base. This causes these groups of fibres to rest on each other in the unexpanded genital bulb and form folds. The folds completely disappear in the course of expansion. Bearing these factors in mind, it is postulated that the hematodocha is not simply a membrane composed of a random arrangement of chitinous, non-elastic and elastic fibres, but that this arrangement is the only factor that causes swivelling of the bulbus in a predetermined direction (that is anticlockwise for the right pedipalp and clockwise for the left one when viewing their tarsal organs ventrally).

It seems that the retractor ligament plays a major role in the swivelling action. If we look at fig. 16*a* we see that as the bulbus is pushed up by increasing blood pressure, point X of the subtegulum will tend to move towards the ectal side of the cymbium due to the restriction in its movements caused by the retractor ligament. Once the petiole is against the ectal rim of the cymbium, point X cannot be pushed out any further. However as the haemolymphatic pressure increases the folds of the long hematodochal portion will tend to expand to their maximum. By now the bulbus is raised out of the alveolus and as the folds of the long hematodochal portion (whose distal end is attached to the ectal side of the tegulum) expand, this will pull the ectal side of the tegulum to the ental side of the cymbium in a swivelling motion across the posterior part of the alveolus. In this process point X will pivot on the distal ends of the retractor ligament and point O will now be on the ectal side of the cymbium. This would be the stage prior to that shown in fig. 16*b*. Further increase in haemolymphatic pressure will cause maximal expansion of the long hematodochal portion and this in turn will push the whole bulbus more to the ectal side of the cymbium as shown in fig. 16*b*.

The importance of the arrangement of the various types of fibres or strands of the hematodocha, as described, now becomes apparent. If the pseudohelical arrangement of the primary fibres in a certain path together with the mechanism described was not present, then when the expansion of the hematodocha reaches the stage where point X pivots on the distal end of the retractor ligament, there would be nothing to prevent the bulbus from swivelling in a clockwise direction instead of the anticlockwise swivelling observed (for the right tarsal organ viewed ventrally).

Bearing all this in mind, we can hence ascribe the following functions to the various components of the basal hematodocha of the tarsal organ of *P. castaneus*:

(i) The retractor ligament

(*a*) Due to its shortness and resistance to stretch it is the prime cause of the swivelling movement of the whole bulbus during expansion.

(*b*) It anchors the ental boss region of the subtegulum on the ectal side of the cymbium.

(*c*) Owing to its general stiffness it will also tend, to a small extent, to bring the bulbus back into the alveolus after copulation. Hence its name.

(ii) The pseudohelical arrangement of the hematodocha

(*a*) It controls the direction of the swivelling movement in expansion and is thus important in ensuring movement of the embolus in the right direction initially.

(*b*) It controls subsequent movement of the embolus tip towards the dorsal region of the tibial apophysis and hence the atrium and bursa copulatrix of the female in copulation. This function and (*a*) above are reversible, that is, it will direct the bulbus back to its resting position after copulation.

(*c*) The non-elastic chitinous fibres of the hematodocha will put a limit to its expansion, that is, until all portions of the hematodocha are unfolded.

(*d*) The elastic fibres will cause refolding of the hematodochal membrane when blood pressure drops after copulation and it is probably the prime cause of such action.

(iii) The petiole

(*a*) It confers added rigidity to the retractor ligament of which it is a part.

(*b*) It protects the retractor ligament from injury when pressed and pushed against the ectal rim of the cymbium.

(*c*) Its petiolar corrugations probably confer added strength, preventing too much bending, in the same way as corrugations prevent bending of a flat metal panel.

5. *Degree of complexity of the palpus of P. castaneus*

Levi (1961) made an attempt to show that the sclerites in the tarsal organs of male spiders probably evolved as functional structures and were retained after losing their functions. He states in his conclusion that: 'Unfortunately, it cannot be known with certainty what constitutes primitive or highly evolved characters, although the assumption has been made here that the primitive palpus was simple.'

Comstock (1910) divides the tarsal organs of male spiders into three main groups according to their degree of complexity:

(1) The simple type of palpi, e.g. *Filistata*, *Loxosceles*, the tarantulas (Mygalomorpha) *Hypochilus* and *Dysdera*.
(2) The intermediate type of palpi; e.g. *Atypus*, *Pachygnata* and *Tetragnatha*.
(3) The more specialized type of palpi, e.g. *Linyphia* and a maximum degree of specialization in *Aranea*.

It is not within the scope of this investigation to discuss comparatively the position of *P. castaneus* in this general scheme, but it will suffice to state that its palpus, in degree of complexity, would be situated between the intermediate and more specialized forms.

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VIII REFERENCES

Alexander, A. J. & Ewer, D. W., 1957. On the origin of mating behaviour in spiders. *Amer. Nat.* **91** (860): 311–317

Barnes, R. D., 1966. *Invertebrate Zoology*. Pub. W. D. Saunders Company.

Barrows, W. M., 1925. Modification and development of the arachnid palpal claw, with especial reference to spiders. *Ann. ent. Soc. Amer.* **18**: 483–516.

Berland, L., 1932. Les Arachnides (scorpions, Araignées, etc.) Biologie Systématique. *Encycl. ent.* (A) **16**: 1–485.

Blauvelt, H. H., 1936. The comparative morphology of the secondary sexual organs of *Linyphia* and some related genera, including a revision of the group. *Fests. Prof. Dr. Strand.* **2**: 81–171.

Bristowe, W. S., 1926. The mating habits of British Thomisid and Sparassid spiders. *Ann. Mag. nat. Hist.* **18** (9): 114–131.

——— 1929. The mating habits of spiders, with special reference to problems surrounding sex dimorphism. *Proc. zool. Soc. Lond.* **2**: 309–358.

——— 1930. A supplementary note on the mating of spiders. Ibid. **2**: 395–413.

——— 1931. The mating habits of spiders: A second supplement with the description of a new Thomisid from Krakatau. Ibid. Part 4: 1401–1412.

——— 1958. *The world of spiders*. Publ. Collins, London.

Fronte Gatenby, J., and Painter, T. S., 1946. *The microtomist vade-mecum*. J. & A. Churchill Ltd., London.

Carleton, H. M., 1938. *Histological technique*. Oxford Univ. Press.

Comstock, J. H., 1910. The palpi of male spiders. *Ann ent. Soc. Amer.* **3** (3): 161–185.

Cooke, J. A. L., 1966. Synopsis of the structure and function of the genitalia in *Dysdera crocata* (Araneae, Dysderidae). *Senck. biol.* **47** (1): 35–43.

——— 1969 (1970). Spider genitalia and phylogeny. *Bull. Mus. Nat. Hist. nat.*, 2^{e} ser., **41**, suppl. 1: 142–146.

Crowe, W., 1951. Die grobe Morphologie des männlichen Genitalapparates einiger Araneen. *Dent. zool. Zeit.* **1** (3): 169–186.

——— 1954. Beschreibung, Morphologie und Lebensweise der *Eucta kaestneri* sp. n. (Aranae, Tetragnathidae). *Zool. Jahr Syst.* **82**: 425–452.

Exline, H. E. & Witcomb, W. H. 1965. Clarification of the mating procedure of *Peucetia viridans* (Araneida: Oxyopidae) by a microscopic examination of the epigynal plug. *Flor. Ent.* **48** (3): 169–171.

Gerhardt, U., 1923. Weitere sexualbiologische Untersuchung an Spinnen. *Arch. f. Naturgeset.* **89** (10 a): 1–225.

——— 1928. Biologische Studien an griechischen, corsichen und deutschen Spinnen. *Morph. Oekol Tiere.* **10** (4): 576–675.

——— 1933. Neue Untersuchungen zur Sexualbiologie der Spinnen, insbesondere an Arten der Mittelmeerlaender und der Tropen. Ibid. **19**: 185–227.

Gering, R. L., 1953. Structure and function of the genitalia in some American agelenid spiders. *Smiths. misc. Coll.* **121** (4): 1–84.

Grasse, P., 1949. *Traité de Zoologie, anatomie, systematique, biologie.* Tome VI. Masson & Cie.

Grasshoff, M., 1964. Die Kreuzspinne *Araneus pallidus*—ihr Netzban und ihre Paarungsbiologie. *Natur und Museum* **94** (8): 305–314.

Gurr, G. T., 1952. *Biological staining methods*. 5th edition. Publ. G. T. Gurr Ltd., London.

Harm, M., 1934. Bau, Function und Entwicklung des akzessorischen Kopulationsorgans von *Evarche marcgravi* Scop. *wiss. Zool.* **146**: 123–134.

Helsdingen, P. J. van, 1965. Sexual behaviour of *Lepthyphantes leprosus* (Ohlert) (Araneida, Linyphiidae), with notes on the function of the genital organs. *Zool. Med.* **41**: 15–42.

——— 1969. A reclassification of the species of *Linyphia* Latreille based on the functioning of the genitalia (Araneida, Linyphiidae), I. *Zool. Verh.* **105**: 1–303.

Homann, H., 1935. Die Function des maennlichen spinnentaster in Versuch. *Zool. Anz.* **109**: 73–75.

——— 1949. Blutdruck und Haütung bei Spinnen. *Die Naturwissenschaften.* **36** (1): 21–24.

Humason, G. 1962. *Animal tissue technique*. W. H. Freeman and Company.

Lawrence, R. F., A conspectus of South African spiders. *Dept. agr. techn. Serv. Sci. Bul.* No. 369. (Plant protection series).

Levi, H. W., 1961. Evolutionary trends in the development of palpal sclerites in the spider family Theridiidae. *J. Morph.* **108** (1): 1–10.

Merret, P., 1963. The palpus of male spiders of the family Linyphiidae. *Proc. zool. Soc. Lond.* **140** (1): 347–467.

Montgomery, T. H., 1903. Studies on the habits of spiders, particularly those of the mating period. *Proc. Acad. nat. Sci. Phil.* **55**: 59–149.

MUMA, M. H. & STONE, K. J., 1965. Activations of spider spermatozoa. *Ent. News.* **76** (7): 177–178.

OSTERLOH, A., 1922. Beitraege zur Kenntnis des Kopulations-apparates einiger Spinnen. *Zeits wiss. Zool.* **119**: 326–421.

PANTIN, C. F. A., 1964. *Notes on Microscopical technique for Zoologists.* Cambridge Univ. Press.

PARRY, D. A., 1957. Spider leg-muscles and the autotomy mechanism. *Quart. J. micr. Sci.* **98** (3): 331–340.

——— 1959. The hydraulic mechanism of the spider leg. *J. exp. Biol.* **36** (2): 523–433.

——— 1960. Spider hydraulics. *Endeavour* **19** (75): 156–162.

PEACOCKS, H. A., 1950. *Elementary microtechnique.* 2nd edition. Edward Arnold & Co. London.

PETRUNKEVITCH, A., 1911. Sense of sight, courtship and mating in *Dugesiella hentzi. Zool. Jahrb. Anat.* **31**: 355–376.

PUSEY, H. K., 1939. Methods of reconstruction from Microscopic sections. *J. Roy. micr. Soc.* **59** (3): 232–244.

RICHARDS, A. G. & KORDA, F. H., 1948. Studies on Arthropod cuticle. II Electron microscope studies of extracted cuticle. *Biol. Bull.* **94** (3): 212–235.

RICHARDS, A. G., 1953*a*. Notes and News in Entomology. Some notes on the role of epidermis in insects and ticks. *Ent. News.* **64** (3): 73–76.

——— 1953*b*. Structure and development of the integument. Reprint from *Insect Physiology.* Publ. John Wiley & Sons.

ROVNER, J. S., 1966. Courtship in spiders without prior sperm induction. *Science,* **152** (3721): 543–544.

——— 1967. Copulation and sperm induction by normal and palpless male Linyphiid spiders. Ibid. **157** (3790): 835.

——— 1971. Mechanism controlling copulatory behaviour in Wolf spiders (Araneae: Lycosidae). *Psyche* **78** (3): 150–165.

SAVORY, T., 1964. *Arachnids.* Academic Press. London & N.Y.

SIMON, E., 1892–1895. *Histoire naturelle des araignées.* Tome 1 (Deuxième édition) pp. 1–1084. Librairie Encyclopedique de Roret.

SUOMALAINEN, K. U., 1964. Histological studies on the poison glands of Araneids. *Ann. zool. Fenn.* **1**: 89–93.

SZOMBATHY, C. VON, 1915. Uber Bau und Funktion der männlichen Kopulationsorgane bei *Agalena* und *Mygale. Ann. Hist.-nat. Mus. Nat. Hungarici* **13**: 252–276.

WARREN, E., 1923. Note on a lizard-eating South African spider *Ann. Natal. Mus.* **5** (1): 95–100.

——— 1928. The comparative histology of the testis and the origin of the spermatozoa in certain South African spiders. Ibid. **6** (1): 1–88.

WIGGLESWORTH, V. B., 1959. A simple method for cutting sections of chitin, and for cutting sections in the 0,5 to 1μ range. *Quart. J. micr. Sci.* **100** (2): 315–320.

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